PATHOGEN POTENTIATION AND IMMUNODEPRESSION IN TICK-BORNE FEVER

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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ABSTRACT

The clinical, parasitological and haematological parameters of tick-borne fever (TBF) in sheep were assayed. Infections were characterised by incubation periods of one to four days followed by fevers which lasted from four to 12 days. The prepatent periods ranged from two to seven days and the visible parasitaemias persisted for six to ten days. Other clinical signs were minimal. Haematological changes, however, were dramatic; an initial leucocytosis was followed by a profound leucopaenia attributable first to a fall in lymphocyte and eosinophil numbers and later to a neutropaenia.

The nature of the TBF-induced lymphocytopaenia was investigated and found to be associated with a significant decrease in the number of peripheral B-lymphocytes and with a small reduction in the number of T-lymphocytes.

Evidence of immunosuppression was sought and found by measuring the primary and secondary antibody responses of TBF-infected sheep to a commercial clostridial vaccine. Injections of the vaccine elicited primary and secondary serum antibody responses in both TBF-infected and normal sheep but the antibody titres in the infected sheep were observed to be significantly lower than the titres in the normal sheep. In contrast to the suppressed humoral immune responses the cell-mediated response as measured by a delayed skin hypersensitivity reaction to dinitrochlorobenzene was unimpaired in the TBF-infected sheep.

The effect of TBF on phagocytosis by neutrophils was examined. The phagocytosis of staphylococci was considerably reduced during

the TBF parasitaemia; this reduction was attributed to the limited phagocytic activity of the parasitised neutrophils.

Tick-borne fever affected the nasal carriage of Pasteurella haemolytica in sheep; the rate of isolation of the organism from swabs of the nasal secretions increased during the reaction to Cytoecetes phagocytophila infection. The attempt to produce pyaemia in lambs by superimposing TBF on an already existing naturally acquired staphylococcal infection was unsuccessful. On the other hand, concurrent infections of sheep with C. phagocytophila and parainfluenza-3 virus produced respiratory distress and deaths. The exacerbation of the respiratory disease in the animals with dual infections was associated with a suppressed antiviral antibody response.

It is postulated that the observed potentiation of concurrent infections stems from the combined effect of the neutrophilic malfunction and immunosuppression induced by C. phagocytophila

LIST OF ABBREVIATIONS

BPF	bovine petechial fever
cAMP	cyclic adenosine 3', 5' - monophosphate
Con A	concanavalin A
CPE	cytopathic effect
DNCB	2, 4-dinitrochlorobenzene
DPX	distrene 80, plasticiser, xylene
DSH	delayed skin hypersensitivity
EDTA	ethylenediamine tetra-acetic acid
E-rosettes	erythrocyte-rosettes
FITC	fluorescein isothiocyanate
g	(acceleration due to) gravity
HA	Haemagglutination
HBSS	Hanks' balanced salt solution
HI	haemagglutination-inhibition
IBDV	infectious bursal disease virus
IgA	immunoglobulin class A
IgE	immunoglobulin class E
IgG	immunoglobulin class G
IgG_1	immunoglobulin G subclass one
IgG_2	immunoglobulin G subclass two

IgG_3	immunoglobulin G subclass three
IgM	immunoglobulin class M
IHA	indirect haemagglutination
l	litre
LCM	lymphocytic choriomeningitis
LTC	lamb testes cell
MHV	mouse hepatitis virus
µl	microlitre
ml	millilitre
mm	millimetre
mm^2	square millimetre
NCD	Newcastle disease
NSD	Nairobi sheep disease
PBL	peripheral blood lymphocyte
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
PI-3	parainfluenza-3
PMN	polymorphonuclear
PNA^+	peanut agglutinin-positive
RBC	red blood cell
RPV	Rowson Parr virus

SIg^+	surface immunoglobulin-positive
SRBC	sheep red blood cell
TBF	tick-borne fever
$TCID_{50}$	50 percent tissue culture infectious dose
ULV	urethane-induced lymphoma virus
VN	virus neutralisation

INTRODUCTION

Many disease syndromes result from the interactions of the host with several extrinsic agents albeit one of the agents is the aetiological trigger. Recently, an avalanche of reports describing situations in which micro-organisms were shown to enhance the severity of concurrent infections or to increase the susceptibility of the host to other diseases has occurred. Most of these studies demonstrated that infections depressed the host's ability to respond to various antigens and mitogens, and this type of immunosuppression has been claimed to be the factor behind the increased susceptibility of the infected animals to other diseases (Notkins, Mergenhagen and Howard, 1970; Schwab, 1975).

Tick-borne fever (TBF) is a disease of ruminants caused by a rickettsia, Cytoecetes phagocytophila, that often occurs in association with other diseases of sheep such as louping-ill. MacLeod and Gordon (1932), for example, demonstrated that under natural conditions of infection louping-ill and tick-borne fever, which are both transmitted by the tick, Ixodes ricinus, developed concomitantly in the same animal and that the rickettsia triggered the development and aggravated the severity of louping-ill possibly by facilitating the invasion of the central nervous system by the virus. The link between TBF and tick pyaemia is also widely accepted and has been attributed to the neutropaenia induced by C. phagocytophila (Foggie, 1956; 1957). Immunologists now implicate lymphocytes in immunosuppression; there is a lymphocytopaenia in TBF and the rickettsia is also known to produce gross and histological lesions

in the spleen and lymph nodes (Hudson, 1950). My working hypothesis, therefore, was that the causal agent of TBF by inducing lymphocytopaenia suppresses immune responses and, in so doing, predisposes the host to other infections or increases the severity of the reaction.

CHAPTER ONE

REVIEW OF LITERATURE

IMMUNOSUPPRESSION

During the past decade, a considerable amount of information has emerged about the effect of parasitic infections on the function of the immune system. Recent reviews include those of Notkins and his colleagues (1970) and Schwab (1975). Most reports deal with depressed antibody responses to unrelated antigens, a phenomenon well recognised in virus infections. Cremer, Taylor and Hagens (1966) for example, observed that the ability of rats to produce antibody to sheep red blood cells (SRBC) was decreased if the rats were infected at birth with the Maloney leukaemia virus. Subsequently, other workers showed that infection with other leukaemia viruses also resulted in suppressed immune responses to unrelated antigens. Salaman and Wedderburn (1966) reported that the primary and secondary antibody responses to SRBC were depressed in mice infected previously with Friend leukaemia virus. The number of IgM and IgG antibody-forming cells in the spleen were reduced when the virus was injected with or before the antigen. The timing of the virus infection in relation to immunisation was critical since the greatest immunosuppression occurred when the mice were infected before immunisation (Okada, Ishii, Yamaura and Yamamoto, 1966; Wedderburn and Salaman, 1968; Ceglowski and Friedman, 1968). Similarly the suppressive effect of Rauscher leukaemia virus on the primary and secondary antibody responses of mice to SRBC was demonstrated by several workers (Siegel and Morton, 1966; Wedderburn and

Salaman, 1968; Ceglowski and Friedman, 1968; Borella, 1969).

Studies on the immunosuppressive action of non-oncogenic viruses such as the lymphocytic choriomeningitis (LCM) virus gave results similar to those produced with the leukaemia viruses; LCM-infected mice were observed to have fewer antibody-forming cells and produced less antibody to unrelated antigens (Mims and Wainwright, 1968). Infection with the infectious bursal disease virus (IBDV) reduced significantly the antibody responses of chicks to Newcastle disease virus and infectious bronchitis virus vaccines; suppression of the humoral antibody response was attributed to the selective and severe destruction of bursa-dependent lymphoid tissues by IBDV (Faragher, Allan and Wyeth, 1974; Giambrone, Eidson and Kleven, 1977; Giambrone, 1978).

Viruses, furthermore, have been shown to affect cell-mediated immune responses. Delayed skin-graft rejection has been described in mice infected with the lactic dehydrogenase virus (Howard, Notkins and Mergenhagen, 1969) and Gross leukaemia virus (Dent, Peterson and Good, 1965). Kantzler and his co-workers (1974) reported that infection in man with influenza virus resulted in depressed cutaneous delayed hypersensitivity reaction to tuberculin and suppressed phytohaemagglutinin (PHA) response. It was suggested that the capacity to engage in delayed hypersensitivity reaction was correlated with the degree of *in vitro* lymphocyte blast transformation induced by PHA (Oppenheim, 1968). Other investigators have shown that lymphocytes from infants with congenital rubella (Olson, Dent, Rawls, South, Montgomery, Melnick and Good, 1968) and from patients with infectious hepatitis (Mella and Lang, 1967; Willems, Melnick and

Rawls, 1969) were markedly depressed in their ability to undergo blast transformation. Measles virus has been found to inhibit the capacity of lymphocytes to respond to PHA stimulation *in vitro* (Sullivan, Barry, Albrecht and Lucas, 1975) and to suppress T-helper cell function *in vivo* (McFarland, 1974).

The many hypotheses to explain the mechanism of virus-induced immunosuppression were crystallised by Notkins and his colleagues (1970). Viruses which infect or replicate in the lymphoreticular tissues depressed antibody production by altering the uptake and processing of antigens, by depressing antibody synthesis, and/or by destroying antibody-forming cells or their precursors, whereas, with the leukaemia viruses the transformation of antibody-precursor cells into neoplastic cells decreased the total number of cells capable of producing antibodies. Other factors such as competition between the infecting virus and immunising antigen for noncommitted antibody-producing cells or the release of endogenous adrenocortical hormones during the course of virus infections were also suggested.

Immunosuppression by infectious organisms is not restricted to viruses; bacteria as well as protozoan and metazoan parasites have been shown to induce immunosuppression. Malakian and Schwab (1968; 1971) demonstrated a membrane-associated immunosuppressant in group A *Streptococcus* and reported that this bacterial component suppressed antibody-forming cells and also stimulated certain T-cell responses in mice. It was postulated that this membrane-associated immunosuppressant functioned by stimulating the suppressor T-cell population. Floersheim and his colleagues (1971) showed that injection of a crude extract of disrupted *Pseudomonas aeruginosa*

inhibited markedly the tuberculin reaction in guinea pigs and prolonged skin homograft survival in mice; the phenomenon was found to be dose dependent. The mechanism involved was not clear but it was suggested that it was entirely on the effector side in the allograft response. Employing a model of delayed hypersensitivity and granuloma formation in response to Schistosoma mansoni eggs, Warren and his co-workers (1974) observed that cholera enterotoxin produced a more effective suppression of cell-mediated immunity than the anti-lymphocyte serum. Results of their studies showed that cholera enterotoxin increased the level of cyclic adenosine 3', 5'-monophosphate (cAMP) in splenic white cells and also decreased the number of splenic white cells and of peripheral lymphocytes suggesting that the enterotoxin functioned through selective destruction of lymphocytes or by affecting their distribution. The work of Kipatovskii and Stanislavski (1971) drew attention to the probable immunosuppressive action of bacterial endotoxin. They demonstrated that injection of cell-free extracts from Escherichia coli prolonged the skin allograft survival and also suppressed antibody formation against SRBC and Vi antigen in mice if the extract were given before the antigens.

Recent studies on Mycoplasma pneumoniae infection of man and hamsters (Liu, Jayaneta, Voth, Muangmanee and Cho, 1972), M. arthritidis infection of rats and mice (Kaklamanis and Pavlatos, 1972), M. mycoides infection of cattle (Roberts, Windsor, Masiga, Kariavu, 1973) and M. suipneumoniae of pigs (Adegboye, 1978) indicated that these infections could result in a suppression of the host's immune responses at least during the active stage of the disease. In these

studies, the cell -mediated immunity was found to be selectively depressed as there were no reports of suppression of humoral antibody response except in M. arthritidis infection where both types of immune response were apparently depressed. The mechanism of mycoplasma-induced immunosuppression remains unclear. Adegboye (1978) proposed that the immunosuppression induced by mycoplasmas was probably due to a paucity of sensitised lymphocytes in the peripheral blood during the acute stage of the infection, a hypothesis which was supported by reports that M. arthritidis infection in mice was associated with a lymphopaenia (Edwards, Deiss, Cole and Ward, 1975) and that the percentage of T-lymphocytes in the peripheral blood was decreased during acute mycoplasmal pneumonia in man (Niklasson and Williams, 1974).

Immunosuppression has been associated with protozoal infections, the best-studied examples being experimental infections of small laboratory animals with trypanosomes and plasmodia. The studies of Salaman and his colleagues (1969) were the first to demonstrate that Plasmodium berghei had an immunosuppressive effect. They observed that mice infected with P. berghei had a markedly diminished response to SRBC injected during the period of parasitaemia. Greenwood and his colleagues (1971) found that mice infected with P. berghei had normal skin homograft rejection reaction and other cell-mediated immune responses. Natural infection of man with P. falciparum was also noted to lower the antibody responses to Salmonella typhi O antigen and tetanus toxoid (Greenwood, Bradley-Moore, Palit and Bryceson, 1972). In an attempt to elucidate the nature of the immunosuppression associated with malaria, Krettli and Nussenzweig

(1974) investigated the cellular composition of the lymphoid organs during the course of P. berghei infections in mice. Their main findings were a progressive and profound reduction in thymus weight and number of T-cells early in the reaction, and a reduction in the number of both T- and B-cells later in the infection, with an increase of a 'null' cell population. It was postulated that the mechanism of antibody suppression in malaria stemmed from altered macrophage function since irradiated mice were reconstituted with spleen cells from P. berghei-infected mice (Greenwood et al., 1971; Loose, Cook and Diluzio, 1972; Tanabe, Waki, Takada and Suzuki, 1977; Lelchuk, Taverne, Agomo and Playfair, 1979).

There has been considerable evidence that trypanosomes cause immunosuppression in man, laboratory animals and ruminants (Goodwin, 1970; Goodwin, Green, Guy and Voller, 1972; Greenwood, Whittle and Molyneux, 1973; Urquhart, Murray, Murray, Jennings and Bate, 1973; Holmes, Mammo, Thomson, Knight and Lucken, 1974; Mackenzie, Boyt, Emslie, Lander and Swanepoel, 1975; Sollod and Frank, 1979). Greenwood and his colleagues (1973) reported that the induction and expression of cell-mediated immunity and the humoral responses were all impaired in patients with trypanosomiasis. Infection with trypanosomes has been found to depress the humoral antibody response of cattle and sheep to bacterial vaccines (Holmes et al., 1974; Mackenzie et al., 1975) and of cattle and mice to viral vaccines (Scott, Pegram, Holmes, Pay, Knight, Jennings and Urquhart, 1977; Whitelaw, Scott, Reid, Holmes, Jennings and Urquhart, 1979; Reid, Holmes and Skinner, 1979). Urquhart and his co-workers (1973) demonstrated that in rats in which Nippostrongylus brasiliensis

infection was superimposed on a previously existing T. brucei infection, the normal process of immune expulsion of adult worms did not occur and the production of protective antibodies was significantly impaired.

Attempts have been made to relate impaired immune responses to changes in the cellular elements of the lymph nodes and spleen. In mice infected with T. brucei, Murray and his colleagues (1974) observed a generalised enlargement of the lymph nodes and gross splenomegaly associated with marked increase of reticulo-endothelial activity and a dramatic proliferation of lymphoblast and plasma cells during the first two to five weeks of infection and a progressive destruction of plasma cells during the later stage of the infection. On histological examination, they found that the B-cell population was grossly altered with a massive plasma cell response in the lymph nodes and spleen. Moulton and Coleman (1977) suggested that the excessive proliferation of plasma cells which disrupted the thymus-dependent areas of lymphoid tissues resulted in interference of T- and B-cell co-operation. Immunosuppression in trypanosomiasis has been attributed to clonal exhaustion of antigen-responsive B-lymphocytes caused by an undefined blastogenic stimulus from the parasite (Hudson, Byner, Freeman and Terry, 1976). Suppressive T-cells and macrophages inhibiting B-cell function were generated during the infection (Corsini, Clayton, Askonas and Ogilvie, 1977; Jayawardena and Waksman, 1977). Recently, Clayton and her colleagues (1979) demonstrated that suppressive T-lymphocytes were unlikely to be entirely responsible for the observed changes since immunosuppression also occurred in athymic mice following infection with

T. brucei. Whether the trypanosomes act directly on a variety of cell types or indirectly through a primary target cell remains to be elucidated.

Infection with Toxoplasma gondii has been reported to depress the antibody response of mice to SRBC (Huldt, Gard and Olovson, 1973; Strickland and Sayles, 1977) and to inactivated polio-virus and louping-ill virus vaccines, and Clostridium welchii type D vaccine (Huldt et al., 1973; Buxton, Reid and Pow, 1979; Buxton, Reid, Finlayson and Pow, 1980). The underlying mechanism in T. gondii-induced immunosuppression is not known but the results of a study on the blastogenic response of Toxoplasma-infected mouse spleen cells to T- and B-cell mitogens indicated that both B- and T-lymphocytes were involved (Strickland, Ahmed and Sell, 1975). Huldt and his co-workers (1973) observed depletion of cortical thymocytes and marked decrease in thymus weight in neonatal mice infected with T. gondii and in pronounced infections, the thymus-dependent zones in the lymph nodes and spleen were affected. It was hypothesised that T. gondii infections either dilute out T-cells in the spleen with unreactive cells such as macrophages, deplete the peripheral lymphoid tissues of T-cells, induced non-specific suppressor cells or activate macrophages which depressed T-cell function non-specifically (Strickland et al., 1975).

Immunosuppression has also been associated with trichinellosis. Mice infected with nematode, Trichinella spiralis, were noted to have depressed antibody response to SRBC; the cell-mediated immune response was also affected since allografts survived much longer in T. spiralis-infected mice than in the non-infected animals (Faubert

and Tanner, 1971; 1974; 1975). Immunosuppression was also demonstrated in normal mice treated either with serum of T. spiralis-infected mice or with a saline extract of T. spiralis larvae (Faubert and Tanner, 1975). Cypess and his co-workers (1973) also showed that mice infected previously with T. spiralis had significantly reduced neutralising and complement-fixing antibody titres to Japanese B encephalitis virus than the mice infected with the virus alone. Immunosuppression in trichinellosis was transient and was correlated to the migrating phase of the life cycle of the parasite (Faubert, 1976). Antigenic competition or antigen-induced depression has been proposed by Faubert (1976) as a possible explanation of the immunosuppression associated with trichinellosis.

PATHOGEN POTENTIATION

Few syndromes have single causes; most are manifestations of complex interactions involving two or more potential pathogens. Theoretically, concurrent infections may or may not interact, the interaction if it occurs being expressed as either a decrease or an increase in the host's response. The susceptibility of the host to a given pathogen can also be influenced by concurrent infections. Kaye, Merselis and Hook (1965), for example, observed that concurrent infection of mice with Plasmodium berghei enhanced their susceptibility to infection with Salmonella typhimurium, the mechanism of this enhancement however was not established. Similarly, P. berghei-infected mice were noted to be particularly susceptible to infection during an outbreak of Streptobacillus moniliformis (Greenwood et al., 1971). Salaman and his co-workers (1969) demonstrated that

P. berghei has an immunosuppressive effect; they observed that the murine plasmodium severely depressed immune reactivity during the height of the parasitaemia and that the pathogenicity of murine sarcoma virus (Harvey) and Maloney virus injected at that period was increased. On the other hand, they found that the oncogenic virus, Rowson Parr virus (RPV), enhanced P. berghei infections in mice; nearly all the virus-infected mice died with overwhelming parasitaemia whereas, most of the mice which did not receive the virus recovered. Cox and Wedderburn (1972) investigated the effect of RPV and ULV, a virus isolated from a urethane-induced lymphoma on concurrent Babesia microti infection in mice and obtained similar results, the level and duration of the B. microti parasitaemias were considerably greater in the mice infected with either of the oncogenic viruses than in the mice which were not given the viruses. Rowson Parr virus and ULV were known to be immunosuppressive (Carter, Chesterman, Rowson, Salaman and Wedderburn, 1970).

Decreased resistance to louping-ill virus was found in mice with chronic Trypanosoma brucei by Reid and his colleagues (1979a) The apparent potentiation of louping-ill virus infection was evidenced by elevated virus titres in the plasma and brain, severe encephalitis, a delayed but higher mortality and a lack of haemagglutination-inhibiting antibody to the virus in animals with dual infections. Studies of infection with another protozoa, Toxoplasma gondii, likewise showed potentiation of louping-ill virus in mice which were infected with both agents (Buxton et al., 1980; Reid, Buxton, Pow and Finlayson, 1980). The more intense viral replication, and the delayed and reduced antibody response encountered

in the concurrently infected mice were held responsible for the greater susceptibility (sic) of these animals to the virus infection which in turn was attributed to the immunosuppressive effect of the protozoal parasite. The immunosuppressive action of Toxoplasma gondii and of trypanosomes has been confirmed earlier by several workers (e.g. Huldt et al., 1973; Strickland et al., 1975; Goodwin, 1970; Urquhart et al., 1973; Scott et al., 1977; Whitelaw et al., 1979).

Virus infections also potentiate other viral diseases. Neonatal chicks which had had an attack of IBDV infection, for example, were reported to experience a high incidence and greater severity of Newcastle disease (NCD) and infectious bronchitis virus infections than chicks which had not been exposed to IBDV (Giambrone et al., 1977). Faragher and his colleagues (1974) demonstrated that the destruction of the lymphoid tissues in the Bursa of Fabricius of young chicks by IBDV, not surprisingly, resulted in diminished antibody response to NCD virus, and this consequently increased the susceptibility to challenge with NCD and prolonged the period of virus excretion after NCD infection (Pattison and Allan, 1974). Mims and Wainwright (1968) noted that ectromelia virus was more lethal for mice that had been infected with LCM virus. They ascribed this enhancement to a decreased cellular immune response of LCM virus-infected mice to ectromelia virus infection.

Infections with respiratory viruses often predispose to bacterial infections in the lung. This viral-bacterial interaction in the respiratory tract was demonstrated in the study by Jakab and Dick (1973) on the combined infection of the murine respiratory

tract with Sendai virus and Pasteurella pneumotropica. Sequential infection of the respiratory tract with the virus and the bacteria decreased the ability of the lungs to eliminate viable bacteria. This synergistic effect was demonstrated by an enhanced mortality in the mice infected with both agents. The spontaneous appearance and multiplication of endogenous Pasteurella in the lungs of mice previously infected with Sendai virus further verified the synergism in viral-bacterial interaction observed in the exogenously imposed P. pneumotropica infection. Heddlestone and his co-workers (1962) reported that infection with parainfluenza-3 virus (PI-3) 24 hours prior to exposure to Pasteurella potentiated the pathogenic effects of the bacteria on the lower respiratory tract. The synergistic effect of combined Pasteurella-parainfluenza-3 virus infections had been recognised by several workers (Hetrick, Chang, Bryne and Hensen, 1963; Saunders and Berman, 1964; Baldwin, Marshall and Wessman, 1967; Sharp, Gilmour, Thompson and Rushton, 1978).

Liu and his colleagues (1972) also showed that intranasal inoculation of hamsters with Mycoplasma pneumoniae followed by Diplococcus pneumoniae resulted in severe pneumococcal septicaemia with high mortality. Active multiplication of the mycoplasm in the respiratory tract before pneumococcal infection was essential for potentiation and development of the pneumococcal septicaemia.

Potentiation of mouse hepatitis virus (MHV) infection in mice by the rickettsia, Eperythrozoon coccoides, has been described by Niven and his co-workers (1952) who found that the pathogenicity of MHV was greatly enhanced by concurrent infection with the harmless blood parasite, E. coccoides. Gledhill and his colleagues (1955)

further showed that E. coccoides was ineffective if given more than 24 hours before MHV suggesting that fatal hepatitis occurred when the period of multiplication of the virus coincided with that of an active infection with E. coccoides. Seamer and his colleagues (1961) similarly observed that concomitant infections with LCM virus and E. coccoides resulted in severe hepatitis and greater mortality in mice infected with both agents than in mice infected with the virus alone. As with MHV, potentiation of LCM virus infection was greatest when virus replication occurred during active eperythrozoonosis. Gledhill, Bilbey and Niven (1965) suggested that the increased susceptibility of the Kupffer cells to MHV was possibly due to the accelerated phagocytosis induced by E. coccoides. Baker, Cassell and Lindsey (1971) added that the ability of E. coccoides to suppress interferon response in infected mice could be an important contributing factor to the increased susceptibility of E. coccoides-infected mice to these viruses.

An earlier evidence of the ability of rickettsias to activate other infections was provided by the report of Daubney and Hudson in 1931. While carrying out experiments on the transmission of heartwater they observed an apparent recurrence of Nairobi sheep disease (NSD) in a recovered sheep after an infection with Cowdria ruminantium by ticks known to be free of NSD virus. Subinoculation of the heartwater and NSD-infected blood from this sheep into five NSD-immune but heartwater-susceptible sheep caused a breakdown of NSD immunity in two of the inoculated sheep. More recently, Grønstøl and Øveras (1980) reported that infection with Eperythrozoon ovis predisposed sheep to Listeria monocytogenes septicaemia.

Sheep infected with both organisms developed more pronounced clinical signs and had longer periods of illness and unthriftiness than the sheep infected with either agents alone. The antibody titres to L. monocytogenes were also found to be considerably lower in the sheep with dual infections than in the sheep infected with Listeria alone. The predisposing effect of Cytoecetes phagocytophila to other diseases is well documented but the mechanism underlying the increased susceptibility of C. phagocytophila-infected animals to other infections has not been adequately investigated.

CHAPTER TWO

EXPERIMENT DESIGN

The present study is divided into three parts: part one is concerned with the clinical and haematological parameters of TBF, part two deals with the effect of TBF on the immune function and on phagocytosis by neutrophils in sheep and part three is primarily concerned with the effects of TBF on concurrent infections in sheep.

BACKGROUND

In the course of preliminary studies on the tick transmission of louping-ill, MacLeod (1932) working at the Moredun Institute showed that ticks collected in louping-ill areas were capable of setting up a febrile reaction in sheep, and animals which had recovered from this reaction were not immune to louping-ill. This hitherto undescribed condition became the subject of a further investigation by Gordon, Brownlee, Wilson and MacLeod (1932) who showed that it was a febrile tick-borne infection, immunologically distinct from louping-ill. They labelled the condition 'tick-borne fever'.

Classification

The causative organism of TBF has been classified as a rickettsia on the basis of its intracellular location, association with an arthropod vector and morphological resemblance to the members of the group; the name *Rickettsia phagocytophila* was proposed (Foggie, 1951). Further morphologic studies however suggested that apart from the presence of an arthropod vector, the TBF organism has

little in common with the members of the genus Rickettsia, the small rod-shaped bodies which are typical of the classical rickettsias have not been seen in tick-borne fever and sera from recovered sheep failed to give the Weil-Felix reaction (Foggie, 1962). A middle-ground position of the TBF organism between the genus Rickettsia and the chlamydias was suggested by Tuomi and von Bonsdorff (1966). The TBF organism is classified under the genus Ehrlichia which includes the parasites of the circulating leucocytes (Bergey's Manual of Determinative Bacteriology, 1974). Foggie (1962) proposed that it should be placed in the genus Cytoecetes established by Tyzzer in 1938 because like Cytoecetes microti, the type species, the TBF organism is a tick-borne parasite of the polymorphonuclear leucocytes.

Morphology

The morphologic features of the TBF organism under light (Hudson, 1950; Foggie, 1951) and electron (Tuomi and von Bonsdorff, 1966) microscopy have been detailed. Under the light microscope, various forms of the organism have been seen; the simplest forms were round, or slightly oval dots of slate-blue colour when stained with Giemsa, larger bodies appeared rounded or irregular and about 4.5 x 3.5 microns in size, and other forms appeared to result from the breaking up of larger bodies and consist of a collection of coccoid elements 0.3 to 0.4 microns in diameter. Tuomi and von Bonsdorff (1966) examined thin sections of leucocyte concentrates from a TBF-infected calf and a sheep. They detected TBF-bodies in neutrophils, eosinophils and occasionally in monocytes within cytoplasmic vacuoles. The preparation from sheep suggested the

presence of developmental forms of the organism. The large particles were found to have a cell wall, plasma membrane, cytoplasm and ribosome-like granules and nucleoid. They were often elongated bearing closer resemblance to rickettsia than to chlamydia. The large particles were considered to be the usual form of the organism. The only mode of multiplication of the TBF-agent suggested by their findings was binary fission of the large particles.

Epidemiology

Tick-borne fever has been reported to be enzootic in tick-infected areas in Britain and Northern Europe (MacLeod, 1936; Tuomi, 1966). It was known to occur in areas of the Netherlands where the tick, Ixodes ricinus was also present (Kuil, Molenkamp, Meyer and Meyer, 1971). The organism was also isolated from cattle in Austria (Hinaidy, 1973).

Natural transmission of TBF to susceptible sheep, cattle, goats and deer is by the tick Ixodes ricinus (MacLeod and Gordon, 1933) but in addition, involvement of other ticks in the transmission of TBF in south-west England has been suggested (MacLeod, 1962). MacLeod in 1936 demonstrated the trans-stadial but not transovarian transmission of C. phagocytophila by the tick, Ixodes ricinus. He showed that ticks which became infected in the larval or nymphal stage were capable of transmitting the infection during the next stage of their development whereas, adult female ticks which became infected did not transmit the infection to their progeny.

In addition to sheep and cattle, goats were shown to be susceptible when inoculated subcutaneously with TBF-infected blood and

when exposed to infected ticks (MacLeod and Gordon, 1933). Cytoecetes phagocytophila has been isolated from feral goats (Capra hircus), roe deer (Capreolus capreolus), fallow deer (Dama dama), and red deer (Cervus elaphus); it was suggested that wild ruminants might serve as reservoirs of TBF (McDiarmid, 1965; Foster and Greig, 1969).

Experimental host

A strain of C. phagocytophila has been successfully adapted to normal and splenectomised guinea pigs and splenectomised mice (Foggie and Hood, 1961). Attempts to propagate the TBF organism in developing hen eggs (Hudson, 1950) and tissue culture systems (Thrusfield, Synge and Scott, 1978) were unsuccessful. The cultivation of C. phagocytophila on leucocyte and Rhipicephalus appendiculatus tissue cultures also failed (Snodgrass, 1974).

Pathogenicity

Gordon and his colleagues (1932) first recognised and described the clinical signs of TBF in sheep. The course of the natural infection was characterised by an incubation period that ranged from four to eight days. Fever marked the onset of illness and lasted for about ten days. Febrile sheep appeared dull, listless and often lost weight. Most sheep recovered from uncomplicated TBF infection.

Tick-borne fever as a cause of occasional outbreaks of abortion in ewes has been reported by Jamieson (1950), Stamp and Watt (1950), Littlejohn (1950) and Øveras (1959). The incidence of abortion associated with TBF in sheep was reported to be in the region of

30 percent and in contrast to the low mortality in non-pregnant sheep, mortality in aborting ewes ranged from 20 to 25 percent (Littlejohn, 1950). Stamp and Watt (1950) provided experimental evidence that TBF was capable of causing abortion in susceptible ewes but whether the abortion was due to the specific effect of C. phagocytophila or a sequela to the febrile reaction is not clear. Tick-borne fever has also been associated and shown to cause infertility in rams (Watson, 1964). It was suggested that a haemorrhagic enteritic episode in sheep was partially associated with the thrombocytopaenia of TBF (Foster, Foggie and Nisbet, 1968).

In addition to causing abortion in ewes, TBF has been recognised as a possible predisposing factor in tick pyaemia in young lambs (Taylor, Holman and Gordon, 1941). The first attempt to reproduce pyaemia by subcutaneous inoculation of staphylococci in lambs affected with TBF by Taylor and his colleagues (1941) was met with little success. However, subsequent studies by Foggie (1956; 1957) provided evidence that lambs in the neutropaenia phase of TBF were more susceptible than normal lambs to pyaemia following the intravenous inoculation of staphylococci. Foster and Cameron (1968a) however, failed to detect a close correlation between the TBF neutropaenia and establishment of staphylococcal infection in lambs.

Tick-borne fever had been reported to increase the susceptibility of sheep to streptococci and Listeria monocytogenes infections in Norway (Øveras, 1972; Grønstøl and Ulvund, 1977). Secondary bacterial pneumonia was also occasionally associated with natural and experimental TBF-infection in sheep (Foggie, 1951; Øveras, 1972). It was also observed that the

debilitating effects of TBF might act as a predisposing factor in enterotoxaemia and/or braxy in lambs (Øveras, 1962).

Tick-borne fever in cattle is characterised by moderate to high fever, depression, inappetence and in some animals rapid respiration and coughing (Hudson, 1950; Tuomi, 1967). In milking cows, fever was accompanied by a sudden and marked drop in milk yield (Hudson, 1950; Øveras, 1962). Tick-borne fever was also linked with infertility, early foetal deaths (Venn and Woodford, 1956) and a severe outbreak of abortion in pregnant heifers introduced into grazing areas where TBF was enzootic (Wilson, Foggie and Carmichael, 1964). As in rams, TBF was found to cause impairment of spermatogenesis leading to transient infertility in bulls (Retief, Neitz and McFarlane, 1971). Occasional deaths from TBF with haemorrhagic syndrome were noted by Foggie and Allison (1960) and Wilson *et al.* (1964).

A haemorrhagic syndrome is a feature of bovine petechial fever (BPF) caused by the rickettsia, *Cytoecetes ondiri*, which is claimed to be related to *C. phagocytophila* (Haig and Danskin, 1962; Snodgrass, 1975). The disease appears to be restricted to the highlands of East Africa (Danskin and Burdin, 1963). The course of the infection is characterised by an incubation period ranging from five to 14 days, followed by a febrile phase during which fine petechiae appeared on the mucous membranes; in lactating cows, a drop in milk yield is usually the first indication of illness (Danskin and Burdin, 1963). Danskin and Burdin (1963) reported that natural infection has been diagnosed only in cattle and experimental transmission in sheep and goats produced only a slight

thermal reaction with no other symptoms. Snodgrass, Karstad and Cooper (1975) isolated C. ondiri from bushbucks. They also demonstrated the multiplication of C. ondiri in the bushbuck, impala, wildebeest and Thomson's gazelles following an experimental inoculation.

Jembrana disease is a disease of cattle and buffaloes which first occurred in the district of Jembrana, Bali, Indonesia in 1964 (Budiarso and Hardjosworo, 1976). As with BPF, a haemorrhagic syndrome is also a characteristic feature of this disease; other clinical signs include fever, increased salivation, generalised lymphadenopathy and anaemia. The exact nature of the aetiologic agent is not known but Budiarso and Hardjosworo (1976) found intracellular organisms resembling Rickettsia in Giemsa-stained smears from the lymph nodes and spleen, and in circulating white cells of clinically affected animals.

Post-mortem findings

The most consistent and sometimes the only gross pathological change observed at post-mortem in sheep and cattle affected with TBF was enlargement of the spleen (Gordon et al., 1932; Jamieson, 1947; McEwen, 1947; Hudson, 1950). On histological examination, Hudson (1950) found degenerative changes in the liver and kidneys and changes suggesting depletion of lymphoid tissues in the spleen and lymph nodes.

Pathogenesis

The pathogenesis of TBF is not understood. In TBF there is a leucocytopaenia which is due first to a decrease in the number of

circulating lymphocytes and later to a neutropaenia (Taylor et al., 1941). In addition, there is also a transient but marked thrombocytopaenia (Foster and Cameron, 1968). Purnell and his colleagues (1977) observed decreases in erythrocyte counts, packed cell volumes and haemoglobin levels and slight increase in mean cell volumes during TBF-infection in splenectomised calves. The precise cause of the lymphocytopaenia is still largely unexplained, but the neutropaenia (Taylor et al., 1941) and the thrombocytopaenia (Foster and Cameron, 1968) were considered to be due either to aplasia of the bone marrow or to a depression in bone marrow activity. The onset of the febrile reaction is associated with the appearance of C. phagocytophila in the phagocytic cells and an increase in the number of neutrophils while the end of the febrile phase and visible parasitaemia is accompanied by a profound neutropaenia.

Various developmental forms of C. phagocytophila have been seen and described under the electron microscope, and it was suggested that the uninhibited multiplication of the organism within the granulocytes eventually leads to some physical destruction of the parasitised cells (Tuomi and von Bonsdorff, 1966). Foster and Cameron (1970a) demonstrated that diapedesis by neutrophils containing TBF-inclusion bodies was inhibited.

The distribution of C. phagocytophila in the tissues following inoculation of TBF-infected blood has been studied in sheep. The results of this study indicated that multiplication took place in the lungs within 24 hours and subsequent spread of the organisms to other tissues before the onset of patent parasitaemia suggested

that the visible form of the organism in leucocytes was not the vehicle by which the infectivity was spread throughout the body (Snodgrass, 1974).

The development of latent infection after an apparent recovery from TBF was demonstrated by Foggie (1951). He showed that C. phagocytophila might persist for more than two years. Tuomi (1967) was able to show latency of TBF in cattle by using a large inoculum, 500 ml of blood, taken nine days after the end of the reaction to TBF infection. Hudson (1950) failed to produce infection in cattle with blood collected after 54 days but succeeded in sheep to produce reactions with blood collected 54 days after reaction to the sheep strain.

Immunity

Recovery from natural or experimental TBF-infection was found to confer varying degrees of immunity. Under field conditions, immunity to TBF in a majority of sheep was found markedly lowered after 14 weeks (Øveras, 1962) to six months (Jamieson, 1947) stay in Ixodes-free pastures. Jamieson (1947) suggested that immunity to TBF was probably dependent upon constant repeated infection. Gordon and his co-workers (1932) observed that most sheep were comparatively immune to further infection after recovery from one or repeated inoculations of infected blood. Stamp and Watt (1950) demonstrated that subcutaneous inoculation of ten ml of TBF-infected blood conferred considerable immunity for a period of 12 months. Hudson (1950) proposed that the short-lived immunity observed in the majority of animals was due to a rather unstable premune state that developed during the period after infection which may then be

followed by a period of sterile immunity and a rather short period of complete immunity.

Diagnosis

Tick-borne fever is usually diagnosed by the demonstration of the characteristic TBF-inclusion bodies in granulocytes and occasionally in monocytes in Giemsa-stained blood smears taken during the febrile phase, or by subinoculation of blood into susceptible animals in cases where infected phagocytic cells are no longer detectable under the microscope (Foggie, 1951). The feasibility of using a complement-fixation test for TBF in sheep has been mooted by Snodgrass and Ramachandran (1971).

PART ONE: CLINICAL AND HAEMATOLOGICAL PARAMETERS OF TICK-BORNE FEVER

Eight adult sheep were inoculated intravenously with one ml of a 10^{-1} dilution of blood containing the Old Sourhope strain of *Cytoecetes phagocytophila*. The rectal temperatures were read daily and blood samples in dipotassium ethylenediamine tetra-acetic acid (EDTA) were taken immediately before inoculation and during the reaction at 24-hour intervals. The total and differential white cell counts were determined by standard techniques (Archer, 1965). The course of the TBF parasitaemia was monitored by examining Giemsa-stained blood smears under a light microscope for the presence of the characteristic TBF-inclusion bodies in the phagocytic cells. Two hundred neutrophils were examined in each smear and the number of infected neutrophils per l noted.

Quantitative changes in the lymphocyte subpopulations during the reaction were determined at the same time. Blood for this purpose was drawn from the jugular vein into ten ml evacuated tubes[1] containing preservative-free heparin[2] (10 units/ml), the samples being taken immediately before inoculation and thereafter at weekly intervals. Peripheral blood lymphocytes were isolated from whole blood according to the method described by Jerrells and his colleagues (1980). The B- and T- lymphocytes were identified and enumerated by the method described by Fahey (1980) and Mr. C. Burrells (personal communication).

PART TWO: EFFECT OF TICK-BORNE FEVER ON THE IMMUNE FUNCTIONS AND ON PHAGOCYTOSIS BY NEUTROPHILS IN SHEEP

The study was designed to examine the effect of tick-borne fever on the humoral antibody response to *Clostridium chauvoei* in sheep, to determine the effect of *C. phagocytophila* infection on cell-mediated immunity, and to examine the phagocytic activity of the neutrophils in sheep during the reaction to tick-borne fever.

Evaluation of humoral antibody response to Clostridium chauvoei vaccine

Twenty adult sheep were used. They were susceptible to tick-borne fever and were found to be free of *Cl. chauvoei* agglutinins. The sheep were divided randomly into two groups of ten. One group

[1] Vacutainer, Becton-Dickinson & Co., Rutherford, New Jersey.

[2] Pularin, Duncan, Flockhart & Co. Ltd., London

was injected subcutaneously with one ml of a commercial blackleg vaccine[1] and served as the control. The other ten animals were each inoculated intravenously with one ml of a 10^{-1} dilution of TBF-infected blood and injected subcutaneously with one ml of the vaccine. The animals in both groups were revaccinated subcutaneously with one ml of the vaccine 21 days after the initial vaccination. The sheep were bled for sera immediately before and after vaccination at weekly intervals. The serum agglutinating antibody titres to Cl. chauvoei vaccine were determined by an agglutination test employing standard Cl. chauvoei antigen supplied by Wellcome Research Laboratories.

Delayed skin hypersensitivity test

A delayed skin hypersensitivity (DSH) reaction to a contact sensitising agent, dinitrochlorobenzene[2] (DNCB) was employed to measure cell-mediated immune response in vivo (Turk and Waters, 1969). Twelve adult sheep were divided randomly into three groups of four. The sheep in the first two groups were sensitised with 50 µl of a two percent solution of DNCB in olive oil. Ten days after priming, two of the sheep in each group were inoculated intravenously with one ml of a 10^{-1} dilution of TBF-infected blood. The other two sheep in each group were each given intravenously one ml of normal sheep blood and served as the control. At the onset of the visible TBF parasitaemia all sheep in the first group were

[1]Blackleg vaccine (A.P.), Wellcome, Beckenham, Kent

[2]Phase Separations Ltd., Queensferry, Clwyd

challenged with 10 µl of a 0.2 percent solution of DNCB in olive oil. The sheep in the second group were similarly challenged with DNCB four days after the onset of the TBF reaction.

Two of the sheep in the third group were each inoculated intravenously with one ml of 10^{-1} dilution of TBF-infected blood; two of the remaining sheep in each group were given one ml of normal sheep blood by the intravenous route. All the animals were sensitised with 50 µl of a two percent solution of DNCB in olive oil seven days after inoculation and then challenged ten days after priming with 10 µl of a 0.2 percent solution of DNCB in olive oil.

The animals were examined six hours after challenge to rule out immediate hypersensitivity reactions. The DSH test was read 24, 48 and 72 hours after challenge by measuring the skin-fold thickness of the test and control sites using a dial micrometer[1]. The changes in the skin-fold thickness were estimated by subtracting the value for the control site from that of the test site.

Effect of tick-borne fever on phagocytosis by neutrophils in sheep

The study was carried out with sheep used to measure the clinical and haemtological parameters of tick-borne fever. Blood samples were collected from the jugular vein into 20 ml evacuated tubes containing preservative-free heparin (10 units/ml) immediately prior to inoculation and then three days after the onset of the visible TBF parasitaemia.

[1]Moor and Wright (Sheffield) Ltd., England.

The polymorphonuclear leucocytes were isolated from whole blood according to the method outlined by Phillips and his co-workers (1979). The *in vitro* assay for phagocytosis and intracellular killing of staphylococci by neutrophil leucocytes were carried out according to the method described by Van Furth and his colleagues (1978).

PART THREE: EFFECTS OF TICK-BORNE FEVER ON CONCURRENT INFECTION IN SHEEP

The study was designed to examine the effect of TBF on the nasal carriage of a pathogenic bacterium and on a concurrent virus infection in sheep, and also to examine the effect of *C. phagocytophila* infection on the course of naturally acquired staphylococcal infections in young lambs.

Nasal carriage of Pasteurella haemolytica in sheep with tick-borne fever

Two groups of sheep were used. One group consisted of sheep that were newly bought from a neighbouring farm. Three days after arrival each sheep was examined clinically and two swabs of nasal secretions were collected, one from each nostril. The other group consisted of acclimatised sheep that had been kept indoors for a period of one month. They were sampled 30 days after they were brought indoors. Twenty-five newly bought and 13 acclimatised sheep from which *P. haemolytica* was not isolated were selected.

Sheep in both groups were subdivided into two further groups. Six animals from the acclimatised group were inoculated intravenously

with one ml of a 10^{-1} dilution of TBF-infected blood while the remaining seven sheep were each given one ml of sterile PBS by the intravenous route. Eleven of the newly bought sheep were inoculated intravenously with one ml of the TBF-infected blood and the other 14 sheep were injected intravenously with one ml of sterile PBS and served as the control.

Swabs of nasal secretions were collected immediately prior to inoculation, and three and seven days after the onset of the reaction. The non-infected sheep were also sampled at the same time.

The sheep were bled for sera immediately before inoculation and thereafter at ten days interval.

The isolation and identification of P. haemolytica were done according to the methods decribed by Osbaldiston (1973) and Cowan (1974). The serotypes of the P. haemolytica isolates were determined by an indirect haemagglutination test (Biberstein and Thompson, 1966; Gilmour, personal communication) using specific antisera against the 12 serotypes of P. haemolytica.

Titration of serum antibodies to P. haemolytica was carried out using the indirect haemagglutination test (Biberstein and Thompson, 1966; Gilmour, personal communication) all serum samples being tested against the 12 P. haemolytica serotypes.

Tick-borne fever and concurrent parainfluenza-3 virus infection in sheep

Fifty, six to nine-month-old lambs were divided and allocated randomly into five groups of ten. Group A was inoculated intranasally with two ml of the cell culture-fluid containing approximately

$10^6 TCID_{50}$ per ml of PI-3 virus. Group B was inoculated simultaneously with one ml of the 10^{-1} dilution of TBF-infected blood and the same dose of PI-3 virus by the intranasal route. Lambs in group C were each inoculated simultaneously with two ml of the PI-3 virus suspension and intravenously with one ml of the 10^{-3} dilution of TBF-infected blood. Group D was given one ml of the 10^{-1} dilution of TBF-infected blood and was later inoculated intranasally with two ml of the PI-3 virus suspension at the onset of the TBF parasitaemia. Lambs in group E were each inoculated intravenously with one ml of the 10^{-1} dilution of TBF-infected blood alone.

The lambs were observed thereafter for clinical manifestations of PI-3 virus and C. phagocytophila infections. Rectal temperatures were read daily before blood samples and bilateral nasal swabs were taken. The animals were bled for sera before and after virus inoculation at weekly intervals. The serum antibody titres to PI-3 virus were determined by haemagglutination-inhibition (HI) and virus neutralisation (VN) tests (Grist, Ross and Bell, 1974).

Animals that died during the experimental period were taken to the post-mortem room and were examined for gross lesions. Lung and bronchial lymph nodes were sampled for bacteria and viruses. Representative portions of the lung lesions were taken for histological examination.

The isolation and identification of PI-3 virus from the nasal swabs and lung tissue were done according to the methods described by Grist and his colleagues (1974).

Attempts to produce tick pyaemia in lambs

Eight pairs of twin lambs showing crops of naturally acquired staphylococcal pustules around the lips and on the perineum were selected from a flock which was regularly examined clinically. The lambs were three to four weeks old except for two lambs which were eight weeks old.

One ml of a 10^{-1} dilution of TBF-infected blood was inoculated intravenously into one of each pair, the remaining lamb given intravenously one ml of sterile PBS serving as a control.

The lambs were examined clinically every day. Rectal temperatures were taken and blood samples were collected before and after inoculation at 24-hour intervals. The course of the TBF parasitaemia was monitored by examining Giemsa-stained blood smears. The total and differential leucocyte counts were carried out as described previously in Part One.

STATISTICAL ANALYSIS

Changes in the daily white blood cell counts were determined by taking the rise or fall as percentage of the pre-inoculation level. The percentage values obtained were plotted on a five millimeter grid and linked. The magnitudes of decrease or increase were expressed in terms of the area bounded by the plot. This was achieved by dividing the area into a series of n panels by means of $n + 1$ parallel lines drawn a constant distance apart h. If the lengths of these panels are $Y_0, Y_1, Y_2 \ldots\ldots Y_n$, the area A is given by Trapezium Rule (Hale, 1958):

$$A = h\ 1/2\ (Y_0 + Y_n) + Y_1 + Y_2 + \ldots\ldots + Y_n - 1$$

Since the initial and final values were zero, the area became the total of the individual values multiplied by a constant factor which was determined by the size of the grid, namely, five.

Because the numbers of the eosinophils and monocytes were low relative to the total leucocytes, eosinophil and monocyte data were first tested for symmetry; approximately, one-quarter of the means were found to be derived from asymmeterical data (Snedecor and Cochran, 1967) (Appendix tables 1 and 3). Medians therefore were used in place of means. In contrast, the differences between paired observations were symmetrically distributed (Appendix tables 2 and 4).

The magnitudes of the febrile reaction and parasitaemias were determined in the same manner. The value for the daily rectal temperatures (^{o}C) and the logarithms to the base ten of the absolute numbers of infected neutrophils were plotted on a five millimeter grid and linked. The magnitudes were then expressed in terms of the area bounded by the plot of daily values. The area was itself calculated using the Trapezium Rule. The constant factor used in the temperature plot was 25 and in the parasitaemias, it was 250.

Data were tested for significant differences by the analysis of variance. Differences between or among the means were further analysed either by Student's $\underline{t}$ test or by Duncan's multiple range test. Significant differences between proportions were analysed using the Chi-square test with Yate's correction.

CHAPTER THREE

CLINICAL AND HAEMATOLOGICAL PARAMETERS OF TICK-BORNE FEVER

The features of TBF infection in sheep that were measured and are herein recorded were the febrile reactions, the haematological changes and the visible parasitaemias.

MATERIALS AND METHODS

Rickettsia

The Old Sourhope strain of *C. phagocytophila* was used throughout (Foster and Cameron, 1970b).

Infected donor sheep were bled while exhibiting rising parasitaemias. The blood was collected from the jugular vein into a 50 ml evacuated tube containing heparin (10 units/ml) and then dispensed into two ml aliquots each containing 0.2 ml dimethyl sulfoxide. The mixtures were stored at $-114^{\circ}C$ in the vapour-phase of a liquid nitrogen refrigerator. Immediately prior to use, a cryopreserved aliquot of blood was rapidly thawed in running water and diluted 10^{-1} with sterile phosphate buffered saline (PBS) at pH 7.2. Unused thawed, blood samples were autoclaved and discarded.

Animals

Eight adult sheep were inoculated intravenously with one ml of 10^{-1} dilution of TBF-infected blood. All were found to be susceptible to tick-borne fever.

Observations

All sheep were examined clinically every day. Rectal temperatures were read and blood samples in EDTA were taken before inoculation and during the reaction at 24-hour intervals. Sheep with temperatures of over 40.5°C were considered febrile whereas, the baseline for assessing the magnitude of the febrile reaction was set at 40°C.

Total leucocyte counts were carried out in a Coulter counter[1] and differential white blood cell counts were made on Giemsa-stained blood smears by standard techniques (Archer, 1965). The course of the parasitaemias was monitored by examining Giemsa-stained blood smears under a light microscope for the presence of the characteristic TBF-inclusion bodies in the phagocytic cells. Two hundred neutrophils were examined in each smear and the number of infected neutrophils per 1 noted.

Quantitative changes in the lymphocyte subpopulations during tick-borne fever infection were determined at the same time. Blood for this purpose was drawn from the jugular vein into 10-ml evacuated tubes containing preservative-free heparin (10 units/ml), the samples being taken immediately before inoculation and thereafter at weekly intervals.

Isolation of peripheral blood lymphocytes

Peripheral blood lymphocytes (PBL) were isolated from whole blood according to the method described by Jerrells and his colleagues (1980). The phagocytic cells were first removed by adding

[1]Coulter Electronics Ltd., Dunstable, Bedfordshire

ten ml of blood to 300 mg of carbonyl iron[1] in a universal vial. The mixture was incubated in a water-bath at 37°C for 45 minutes with gentle mixing to encourage contact between the phagocytic cells and iron particles. At the end of incubation, the blood-carbonyl iron mixture was diluted with an equal volume of sterile Hanks' balanced salt solution (HBSS). The lymphocytes were separated from the carbonyl iron-treated blood by layering four ml of the diluted blood over three ml of Ficoll-Paque[2] in sterile centrifuge tubes and centrifuging at 400 g for 35 minutes. The band of mononuclear cells in the Ficoll-Paque plasma interface was collected using a Pasteur pipette, and resuspended in five ml of HBSS containing five units per ml of preservative-free heparin and one percent bovine serum albumin. A strong magnet was used to retain the iron-containing phagocytic cells as the mononuclear cell suspension was poured from one tube to another. Finally the mononuclear cells were washed three times and resuspended in cold HBSS-azide solution (HBSS with five units per ml of heparin, one percent bovine serum albumin and 0.1 percent sodium azide), checked for cell viability and counted in a Coulter counter.

Detection and enumeration of B-lymphocytes

The direct labelling of lymphocyte surface immunoglobulin with pig anti-sheep IgM conjugated with fluorescein isothiocyanate[3] (FITC) was done at 4°C (Fahey, 1980; C. Burrells, personal communication).

[1]Sigma Chemicals Co., St. Louis, MO, USA

[2]Pharmacia, Fine Chemicals AB, Uppsala, Sweden

[3]Eivai Bio Laboratories Ltd., Stoke Poges, Slough, England

A volume of ten µl of the mononuclear cell suspension was added to 100 µl of FITC-conjugated pig anti-sheep IgM and incubated for 60 minutes at 4°C. After incubation, the cells were washed three times and resuspended in three drops of cold HBSS-azide solution. Two coverslip preparations were examined with a phase-contrast/fluorescence microscope[1] with a dry high-power objective (x 40). The number of lymphocytes in the field was counted by phase-contrast; the same field was examined under incident ultraviolet illumination and the number of fluorescing lymphocytes noted. At least 200 lymphocytes were counted in each coverslip preparation and the average number recorded. The number of surface immunoglobulin-positive (sIg^+) lymphocytes was expressed as a percentage of the peripheral blood lymphocytes and also in absolute number.

Detection and enumeration of T-lymphocytes

Peanut agglutinin (PNA), a lectin from Arachis hypogaea was used as a marker of presumptive T-lymphocytes in sheep (Fahey, 1980). The technique was that devised by Mr. C. Burrells (personal communication). A volume of ten µl of the mononuclear cell suspension was added to 100 µl of PNA[2] conjugated with FITC and incubated at 4°C for 60 minutes. The cells were washed three times and resuspended in three drops of cold HBSS-azide solution. The cells were examined twice under coverslips with a fluorescence microscope, counted and averaged in the same manner as the sIg^+ lymphocytes. The number of peanut agglutinin-positive (PNA^+) lymphocytes was expressed as a percentage of the peripheral blood lymphocytes and in absolute number.

[1]Vickers M17 Fluorescence Microscope, Vickers Instruments, Breakfield

[2]Miles Laboratories Ltd., Stoke Poges, Slough, England.

RESULTS

Fever

The onset of illness was indicated by a sudden rise in temperature (Fig. 3.1). The mean incubation period was 2.5 ± 0.5 days in the range two to three days (Table 3.1). A mean peak temperature of 41.5 ± 0.4°C was observed 1.7 ± 0.8 days after the onset of fever which persisted for 6.5 ± 1.4 days in the range four to nine days (Table 3.2). The mean magnitude of the febrile reaction was 1,871.8 ± 586.6 mm^2 (Table 3.2).

Haematology

There was an increase in the total leucocyte counts after inoculation. The leucocytosis, however, was brief and statistically non-significant being immediately followed by a marked and statistically significant leucopaenia (Fig. 3.2, Table 3.3). The total leucocyte counts fell from a pre-inoculation level of 8.2×10^9 ± 1.5×10^9 per l to a mean nadir of 3.0×10^9 ± 0.6×10^9 per l, 8.1 ± 2.5 days after inoculation (Table 3.4).

The differential white cell counts fluctuated markedly in the numbers of the different cell types. The mean lymphocyte count rose significantly from a pre-inoculation level of 5.0×10^9 ± 1.2×10^9 per l to 6.1×10^9 ± 1.4×10^9 per l on the day after inoculation (Fig. 3.2). The mean magnitude of the lymphocytosis was 130.4 ± 118.3 mm^2 (Table 3.5 and Appendix Table 5). The lymphocytosis was of short duration and was followed by a sudden significant drop in the number of lymphocytes (Table 3.2). The mean lymphocyte count reached a mean nadir of 1.3×10^9 ± 0.3×10^9

per l, 5.8 ± 0.6 days after inoculation (Table 3.4). The mean magnitude of the lymphocytopaenia was 2,989 ± 859 mm^2 (Table 3.5 and Appendix Table 5). The lymphocyte counts returned to normal level 11 days after inoculation when the parasitaemia was no longer overt (Fig. 3.2).

There was a dramatic and significant decrease in the number of eosinophils during the reaction (Fig. 3.3, Table 3.6). During the eosinopaenia which lasted two weeks, the eosinophils were not detected for 4.3 ± 0.7 days in the range one to seven days (Table 3.7). The mean magnitude of the eosinopaenia was 4,607 ± 787 mm^2 (Table 3.8 and Appendix Table 6).

The number of neutrophils increased significantly after inoculation (Fig. 3.2, Table 3.3). The mean neutrophil count rose from a pre-inoculation level of 2.8 x 10^9 ± 0.7 x 10^9 per l to a mean maximal count of 3.9 x 10^9 ± 0.7 x 10^9 per l around the time of onset of visible parasitaemia (Table 3.9). The mean magnitude of the neutrophilia was 450 ± 385 mm^2 (Table 3.10 and Appendix Table 7). Subsequently, the neutrophil counts fell precipitously to a mean nadir of 0.4 x 10^9 ± 0.1 x 10^9 per l, 7.3 ± 1.6 days after the onset of visible parasitaemia (Table 3.9). The neutropaenic phase lasted from the eighth to nineteenth day after inoculation and was accompanied by the disappearance of the parasites from the peripheral blood (Fig. 3.2). The mean magnitude of the neutropaenia was 3,598 ± 933 mm^2 (Table 3.10 and Appendix Table 7).

The monocyte counts showed slight increases during the parasitaemia (Fig. 3.3). The rises in monocyte numbers however, were not significant as compared with the pre-inoculation levels

(Table 3.6). The mean magnitude of the monocytosis was 1,955 ± 1,717 mm^2 (Table 3.8 and Appendix Table 8).

Parasitaemia

The prepatent period always lasted two to three days after inoculation (Fig. 3.4, Table 3.1). In 50 percent of the sheep, the incubation period was the same as the prepatent period; in the other sheep, visible parasitaemia preceded fever by no more than one day (Table 3.1). The mean duration of visible parasitaemia was 7.5 ± 0.7 days in the range six to eight days and was similar to the duration of fever (t_{14} = 1.818, P > 0.10) (Table 3.1 and 3.2). The mean magnitude of the parasitaemia was 16,403 ± 1,528 mm^2 (Table 3.11).

High parasitaemias occurred early in the reaction (Fig. 3.4). The mean maximal parasitaemia was $10^{9.20}$ ± 0.1 infected neutrophils per l and was recorded 1.7 ± 1.9 days after the onset of visible paraistaemia (Table 3.11). Relapse parasitaemias were not observed in these sheep.

Detection and enumeration of B-lymphocytes

Peripheral blood lymphocytes bearing surface immunoglobulin were readily identified and enumerated by the method used. Before inoculation, a mean total peripheral blood lymphocyte count of 14.4 x 10^9 per l was recorded (Table 3.12). The number of sIg^+ lymphocytes was 1.6 x 10^9 ± 0.4 x 10^9 per l (Table 3.13). As before, the lymphocytopaenia on day seven after inoculation was statistically significant (t_7 = 4.249, P < 0.010) (Table 3.12). The lymphocytopaenia, seven days after inoculation included a

significant decrease in the number of sIg^+ cells to $0.6 \times 10^9 \pm 0.4 \times 10^9$ per l ($t_7 = 4.786$, $P < 0.010$) (Table 3.13). Fourteen days after inoculation, the mean total peripheral blood lymphocyte count was $11.6 \times 10^9 \pm 2.2 \times 10^9$ per l, with a mean sIg^+ cell count of $2.2 \times 10^9 \pm 0.7 \times 10^9$ per l (Tables 3.12 and 3.13). The total lymphocyte counts showed a return to pre-inoculation levels 21 days after inoculation, the mean count being $14.7 \times 10^9 \pm 1.4 \times 10^9$ per l (Table 3.12). A significant rise in the number of sIg^+ cells to $3.4 \times 10^9 \pm 1.3 \times 10^9$ per l occurred at the same time ($t_7 = 3.929$, $P < 0.010$) (Table 3.13).

The sIg^+ lymphocyte counts were also expressed as percentages of the total peripheral blood lymphocyte counts. Before inoculation, approximately 12 percent of the peripheral blood lymphocytes were found to be sIg^+ cells (Table 3.14). Reduction in the total number of lymphocytes also included a decrease in the percentage of sIg^+ cells seven days after inoculation. As the total lymphocyte counts returned to pre-inoculation levels, the percentage of the sIg^+ lymphocytes rose to 20 percent on day 14 and to 21 percent on day 21 (Table 3.14).

Detection and enumeration of T-lymphocytes

A mean PNA^+ lymphocyte count of $6.3 \times 10^9 \pm 2.1 \times 10^9$ per l was recorded before inoculation (Table 3.15). A slight and non-significant reduction in the PNA^+ cell counts was observed seven days after inoculation, the mean count being $4.2 \times 10^9 \pm 2.5 \times 10^9$ per l. The number of PNA^+ cells returned to the pre-inoculation level of $6.1 \times 10^9 \pm 1.1 \times 10^9$ per l, 14 days after inoculation.

Prior to inoculation, 44 percent of the peripheral blood lymphocytes were found to be PNA^+ cells. The percentage of PNA^+ cells showed only slight changes during the reaction to tick-borne fever (Table 3.14).

DISCUSSION

The quantitative data on the responses of sheep to tick-borne fever were within the range of previously reported figures (Taylor et al., 1941; Foggie, 1951; Foster and Cameron, 1970b; Snodgrass, 1974; Purnell et al., 1977). The effect of different dose levels of TBF-infected blood on the parameters selected for study was not investigated. However, Snodgrass (1974) in a similar study showed by using one ml of 10^0 to 10^{-4} dilutions of blood containing the Old Sourhope strain of C. phagocytophila that with the exception of the prepatent period, the fever and haematological changes were not affected. He found that the prepatent period varied inversely with the dose while the duration of the visible parasitaemias, maximal parasitaemias and day on which maximal parasitaemias occurred were not significantly different between the different dose levels.

The febrile responses were clearly defined. After an incubation period of two to three days, the temperature rose sharply reaching maximal values of 40.9°C to 42.2°C. The duration of the fever was four to nine days. The febrile reactions described were in close agreement with those recorded by Foster and Cameron (1970b) and Snodgrass (1974); they also used the Old Sourhope strain of C. phagocytophila.

Foster and Cameron (1970b) observed that of the parameters they selected for study, the febrile response and the neutropaenia were most likely to become complicated by co-existing infections. Two of their experimental animals developed P. haemolytica infection which prohibited the use of the haematological data from these animals for comparative purposes. They also suspected that the relatively long febrile reaction in some of the sheep was due to an inadvertent infection with louping-ill virus.

The haematological changes described were largely in agreement with those reported by Taylor and his co-workers (1941). Changes in the leucocyte picture were dramatic; the initial leucocytosis was brief and was succeeded by a profound leucopaenia attributable first to a decrease in the number of lymphocytes and eosinophils and later to a neutropaenia.

The lymphocyte reaction included a brief but significant lymphocytosis on the day after inoculation. This phenomenon has not been reported or noted by previous workers (Taylor et al., 1941; Foggie, 1951; Snodgrass, 1974; Purnell et al., 1977). The lymphocytosis was followed by a sudden and significant drop in the number of lymphocytes. During the reaction, the mean lymphocyte count decreased by 72 percent. The lymphocyte counts returned to normal levels 11 days after inoculation. In contrast, in cattle infected with C. phagocytophila, the lymphocytopaenia was short; the lymphocyte count decreased on day six and rose to normal level on day nine (Purnell et al., 1977).

Significant decrease in the eosinophil counts occurred three days after inoculation. The eosinophils showed the greatest

depression. During the eosinopaenia, the eosinophils were not detected for one to seven days. Taylor and his group (1941) noted without comment that the reaction of the eosinophils was essentially the same in sheep that were infected with louping-ill virus and in those inoculated with Staphylococcus aureus. A current hypothesis attributes the eosinopaenia in acute infections as being the result of a massive release of steroids because of stress (Morgan and Beeson, 1971).

The neutrophil reaction was characterised by a post-inoculation neutrophilia similar to that observed by Taylor and his colleagues (1941) and Foggie (1951) in sheep and Purnell and others (1977) in cattle. The neutrophilia occurred around the time of onset of visible parasitaemia. The neutropaenia which followed became evident seven days after inoculation. The drop in the number of neutrophils was precipitous; during the neutropaenia, the mean count decreased by 82 percent. The lymphocyte nadir was significantly earlier than that of the neutrophil ($t_{14} = 2.475$, $P < 0.050$). A similar observation was recorded by Snodgrass (1974). The neutropaenic phase lasted from the eighth to nineteenth day after inoculation and was accompanied by the disappearance of the parasites from the peripheral blood. The neutrophil counts returned to pre-inoculation levels 20 days after inoculation. There was not a significant reactive neutrophilia as described by Taylor and his colleagues (1941). Foggie (1951) also failed to observe a reactive neutrophilia.

The changes in the monocytes were less consistent showing irregular periods of non-significant increases during the reaction.

This observation was in agreement with that of Taylor's group (1941) and Purnell and his co-workers (1977). Snodgrass (1974) on the other hand, claimed to have noted a significant monocytosis on the fifth day of the reaction. Examination of his data however revealed that his method of analysis was invalid.

Previous workers had reported the parasitaemias (e.g. Foggie, 1951) as the percentage of infected neutrophils. This did not take into account the marked changes in the number of neutrophils that occurred during the reaction. By using the logarithms of absolute numbers of infected neutrophils, this limitation was obviated. The characteristic TBF-inclusion bodies were occasionally found in the eosinophils or monocytes and the omission of parasitised eosinophils or monocytes in the computation did not seriously underrate the total parasitaemias.

The prepatent period always lasted two to three days after inoculation. Visible parasitaemia lasted from six to eight days and was similar to the duration of fever. High parasitaemias occurred early in the reactions, maximal parasitaemias ranging from $10^{9.03}$ to $10^{9.45}$ infected neutrophils per l. In cattle, Purnell and his colleagues (1977) demonstrated parasites from day four to day 15 after inoculation with a maximal parasitaemia of 2.68×10^3 infected neutrophils per cu.mm ($10^{9.42}$ infected neutrophils per l) occurring on day five. Relapse parasitaemias similar to those reported by Foggie (1951) were not observed in the present study.

Morphologically, lymphocytes are classified as small and large lymphocytes (Schalm, Jain and Carroll, 1975). It is now recognised that size difference alone is an inadequate criterion for categorising these cells

(Boggs and Winkelstein, 1976). Current concepts indicate that, functionally, lymphocytes belong to two fundamental classes: lymphocytes bearing detectable surface immunoglobulin which on contact with antigen differentiate into antibody-forming plasma cells, and lymphocytes that have no readily detectable surface immunoglobulin which when activated by antigen may act (a) as antigen reactive cells, (b) as effector cells in cell-mediated immunity, and (c) as 'helper' cells by co-operating with the antibody-forming precursor cells in antibody production against thymus-dependent antigens or act as 'suppressor' cells by regulating the magnitude of antibody production (Roitt, Greaves, Torrigiani, Brostoff and Playfair, 1969; Playfair, 1971; Jacobson, Herzenberg, Riblet and Herzenberg, 1972; Cantor and Asofsky, 1972; Gershon, Cohen, Hencin and Liebhaber, 1972; Raff, 1973; Taylor and Basten, 1976; Rocklin, 1976).

The first indirect demonstration of immunoglobulins on the surface of small lymphocytes was made by Sell and Gell in 1965 who used a variety of anti-immunoglobulin antisera to stimulate blast transformation of rabbit peripheral blood lymphocytes. Subsequently, studies using radioiodinated and fluorescein-conjugated anti-immunoglobulin antisera have shown directly the presence of immunoglobulin on the surface of small lymphocytes in mice (Raff, Sternberg and Taylor, 1970), rabbits (Pernis, Forni and Amantes, 1970), chickens (Rabellino and Grey, 1971), man (Grey, Rabellino and Pirofsky, 1971), horses (Banks and Henson, 1973), pigs (Binns and Symons, 1974), cats (Holmberg, Manning and Osburn, 1976) and dogs (Holmberg, Manning and Osburn, 1977). Quantitative studies

indicated that in mice 50,000 to 150,000 molecules of immunoglobulin were present on individual cells that contained detectable surface immunoglobulin (Rabellino, Colon, Grey and Unanue, 1971). The major source of lymphocytes with surface immunoglobulin has been shown to be the bone marrow in mammals or the Bursa of Fabricii in the chicken while most of the surface immunoglobulin-negative lymphocytes were demonstrated to be derived from the thymus (Unanue, Grey, Rabellino, Campbell and Schmidthe, 1971; Rabellino and Grey, 1971). For this reason surface immunoglobulin has been used as a marker for bone marrow-derived or Bursa-derived (B) lymphocytes and the lack of surface immunoglobulin as an indicator of thymus-derived (T) cells (Grey, et al., 1971). In addition, B-lymphocytes may be differentiated from T-lymphocytes by the presence of specific receptors for the C3 component of complement and receptors for the Fc part of complexed immunoglobulin (Bianco, Patrick and Nussenzweig, 1970; Basten, Miller, Sprent and Pye, 1972).

The ability of thymocytes and thymus-derived lymphocytes to bind spontaneously heterologous erythrocytes to form clusters commonly known as E-rosettes has been shown to be a convenient means of identifying T-lymphocytes in man (Jondal, Holm and Wigzell, 1972), dogs (Bowles, White and Lucas, 1975), pigs (Escajadillo and Binns, 1975), and cats (Holmberg et al., 1976). This rosetting phenomenon is non-immunological and occurs only around living cells (Jondal et al., 1972). In mice the theta isoantigen has been used as a specific marker for T-lymphocytes (Raff and Wortis, 1970). This surface isoantigen was found present in all thymus lymphocytes and on a discrete subpopulation of lymphocytes in the peripheral

lymphoid tissues of mice (Raff, 1969).

Unlike mouse lymphocytes, the lymphocyte subpopulations in ruminants are still poorly defined. In cattle, the equivalent of murine B-lymphocytes has been identified by the presence of surface immunoglobulin and receptors for the Fc region of complexed immunoglobulin and receptors for complement (Grewal, Rouse and Babiuk, 1978) while in the sheep, the only established marker is the immunoglobulin present on the surface of 20 to 30 percent of the lymphocytes in the recirculating pool (Heron, Poskitt, Cahill, and Trnka, 1978). The binding of sheep erythrocytes to form E-rosettes has been considered as a possible marker for bovine 'T' lymphocytes (Grewal, Rouse and Babiuk, 1976). Pearson and his colleagues (1979) by using a double labelling technique employing a rhodamine-conjugated goat anti-bovine immunoglobulin and fluorescein-labelled lectins showed that the lectin, PNA, bound almost exclusively to those lymphocytes in the PBL population in which surface immunoglobulin was absent. More recently, Fahey (1980) investigated the ability of PNA to bind to lymphocytes in sheep blood and lymph. He found that when cells from the lymphatic fluid were stained with either PNA or fluorescein-labelled $F(ab')_2$ anti-sheep IgM, or both, the sum of PNA-positive cells and surface immunoglobulin-positive (sIg^+) cells equalled the percentage of positive cells in double labelled preparations, whether counted visually or with a fluorescence-activated cell sorter. He concluded that PNA is an efficient marker of presumptive T-lymphocytes in young and adult sheep.

In the mouse, Rabellino and his co-workers (1971) reported that 15 percent of the peripheral blood lymphocytes contained immunoglobulin on their surface. Moreover, the sIg^+ lymphocytes carried a single immunoglobulin class; 19 percent of the sIg^+ cells in the spleen stained with IgG_2 antisera, 12 percent with anti-IgG_1, one percent with anti-IgG_3, six percent with anti-IgA and ten percent with anti-IgM antisera, and in the blood, lymph nodes and bone marrow the different immunoglobulins were represented in roughly the same proportions except in the blood where the proportion of IgM-containing cells was approximately 30 percent of the total sIg^+ cell population. Grey and his colleagues (1971) noted that in man about 28 percent of the PBL population were sIg^+ and on the average 15 percent possessed IgG, six percent carried IgA and eight percent contained IgM on their surface. In cattle, the percentage of sIg^+ cells in the peripheral blood has been reported to be about 28 to 32 (Grewal et al., 1976; Reeves and Renshaw, 1978; Pearson, Roelants, Lundin and Mayor-Withey, 1979). Using a fluorescein-conjugated pig anti-sheep IgG antisera, Symons and Binns (1975) found that the mean percentage of sIg^+ cells in the blood of one-year-old sheep was 23 percent whereas, in the eight-year-old sheep the mean percentage was 9.7; they observed that pregnancy did not influence these values. Similarly, Takashima and Olson (1980) reported that the mean percentage of sIg^+ cells in the PBL population of normal adult sheep was 24.

Reeves and Renshaw (1978) used the E-rosettes as markers for the T-lymphocytes in cattle and demonstrated that 45 percent of the cells in the PBL population formed spontaneous E-rosettes with

chicken red blood cells. Pearson and his colleagues (1979) also observed that about 45 percent of the lymphocytes in the peripheral blood of cattle bound PNA; these PNA-positive cells were found to be negative for surface immunoglobulin.

Results of the present study showed that in the normal adult sheep the mean percentage of peripheral blood lympnhocytes binding pig anti-sheep IgM was 12. Since the antiserum used was mono-specific for IgM, this number probably represents the percentage of B-lymphocytes bearing IgM on their surface. Fluorescein-conjugated PNA was used as marker for presumptive T-lymphocytes in sheep. Results showed that in the normal sheep 44 percent of the peripheral blood lymphocytes were PNA-positive.

Muscoplat and his colleagues (1973) investigated the number of sIg^+ cells in the PBL population of normal and diseased cattle. In the normal animals 30 percent of the peripheral blood lymphocytes carried surface immunoglobulin whereas, in two calves affected with chronic bovine viral diarrhoea (BVD) only four to 15 percent of the peripheral blood lymphocytes were found to be sIg^+. On histo-logical examination, the lymph nodes from the affected calves were found to be devoid of germinal centres and the area of the cortex being replaced by proliferative reticular cells; the thymus was also depleted of lymphocytes. In another study, Muscoplat and his co-workers (1974) reported that in calves with acute lymphocytic leukaemia less than one percent of the peripheral blood lymphocytes possessed surface immunoglobulin. In contrast, approximately 19 percent of the PBL population in normal age-matched calves demon-strated surface immunoglobulin.

Infectious bursal disease of chickens provides an excellent example of a disease which specifically affects the bursa-derived cells; the disease is characterised by massive destruction of lymphoid tissues in the Bursa of Fabricius, in the germinal follicles and in the periarteriolar lymphoid sheath in the spleen, and minimal changes in the thymus and other thymus-dependent tissues (Cheville, 1967). The lack of effect on T-cell function in chicks infected with IBDV was found to correlate with the histopathological changes (Giambrone, Donahoe, Dawe and Eidson, 1977).

The lymphocytopaenia induced by C. phagocytophila in sheep was found to be associated with a profound, statistically significant decrease in the number of cells bearing surface immunoglobulin but only a small reduction in the number of PNA-positive lymphocytes. A direct interpretation of these results is that the lymphocytopaenia of tick-borne fever results from a depletion in the number of circulating B-lymphocytes. The B-cell lymphocytopaenia in TBF, however, was transient, occurring during the clinical episode. The number of sIg^+ cells soon returned to pre-inoculation levels and even increased significantly within 14 days after inoculation. The increase in the number of sIg^+ cells during the recovery phase was probably an indication of a developing humoral immune response to C. phagocytophila infection. In TBF-infected sheep complement-fixing antibody was detected between 11 and 14 days after inoculation (Snodgrass, 1974). Heron and his colleagues (1978) observed that the number of sIg^+ cells in the lymph of sheep increased after immunisation with heterologous erythrocytes and the increase in the sIg^+ cells paralleled the

plaque-forming cell response.

In summary, the immunodepressive effect of TBF must be associated with the B-cell lymphocytopaenia which suggests that there is a non-specific suppression of antibody production while cell-mediated immune mechanisms are not affected.

Table 3.1 Incubation periods, prepatent periods and duration of visible parasitaemias in eight TBF-infected sheep

Sheep No.	Incubation period (days)	Prepatent period (days)	Duration of visible parasitaemia (days)
291	3	3	6
292	3	3	8
294	2	3	7
295	2	3	7
297	2	3	8
299	3	3	8
319	3	3	8
320	2	3	8
mean	2.5	3.0	7.5
standard deviation	0.5	0.0	0.7

Table 3.2 Maximal fevers, duration and magnitude of the febrile reactions in eight TBF-infected sheep

Sheep No.	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
291	41.3	3	6	1,775
292	41.2	2	7	2,100
294	42.1	1	9	3,175
295	40.9	2	7	1,550
297	42.0	2	7	1,925
299	42.2	2	6	1,725
319	41.4	0	4	1,325
320	41.4	2	6	1,400
mean	41.5	1.7	6.5	1,871.8
standard deviation	0.4	0.8	1.4	586.6

Table 3.3 Means and standard errors of differences from pre-inoculation counts of leucocytes, lymphocytes and neutrophils ($x10^9/l$) in eight TBF-infected sheep.

Days after inoculation	Parasitaemia	Leucocyte	Lymphocyte	Neutrophil
0	-			
1	-	-0.6 ± 0.5[1]	-1.1 ± 0.2[1]**	0.4 ± 0.4[1]
2	-	-0.1 ± 0.6	-0.03 ± 0.5	0.03 ± 0.3
3	+	0.8 ± 0.5	1.9 ± 0.4**	-0.1 ± 0.4*
4	+	3.3 ± 0.5***	3.0 ± 0.4***	0.4 ± 0.4
5	+	3.5 ± 0.4***	3.2 ± 0.4***	0.2 ± 0.3
6	+	3.6 ± 0.5***	3.5 ± 0.4***	0.02 ± 0.4
7	+	4.1 ± 0.5***	3.2 ± 0.4***	0.7 ± 0.4
8	+	4.0 ± 0.6***	2.7 ± 0.4***	1.1 ± 0.4*
9	+	4.0 ± 0.6***	2.3 ± 0.4***	1.6 ± 0.4**
10	+	4.2 ± 0.5***	1.7 ± 0.4**	2.2 ± 0.2***
11	-	3.6 ± 0.6[1]**	1.0 ± 0.5[1]	2.3 ± 0.3[1]***
12	-	3.3 ± 0.6***	1.0 ± 0.5	2.1 ± 0.3***
13	-	3.7 ± 0.5[1]***	1.2 ± 0.6[1]	1.9 ± 0.3***
14	-	3.7 ± 0.4[1]***	1.4 ± 0.6[1]	1.4 ± 0.3[1]***
15	-	3.5 ± 0.5***	1.2 ± 0.4	2.0 ± 0.3***
16	-	2.8 ± 0.5***	0.8 ± 0.4	1.8 ± 0.3***
17	-	2.2 ± 0.5**	0.5 ± 0.4	1.5 ± 0.3**
18	-	2.1 ± 0.3***	0.7 ± 0.3	1.2 ± 0.2**
19	-	1.5 ± 0.4**	0.2 ± 0.3	1.0 ± 0.3**
20	-	0.5 ± 0.6[1]	-0.1 ± 0.4[1]	0.7 ± 0.4[1]

* $P < 0.050$ ** $P < 0.010$ *** $P < 0.001$ [1]Derived from 7 observations

Table 3.4 Total leucocyte count and lymphocyte count nadirs in eight TBF-infected sheep

Sheep No.	Total leucocyte count nadir ($x10^9/l$)	Days after inoculation	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation
291	3.6	8	1.6	6
292	4.0	12	1.6	6
294	3.8	6	1.4	6
295	2.8	4	0.6	5
297	2.7	8	1.7	5
299	2.6	7	1.3	7
319	2.5	10	1.0	6
320	2.5	10	1.0	6
mean	3.0	8.1	1.3	5.8
standard deviation	0.6	2.5	0.3	0.6

Table 3.5 Magnitudes of the lymphocytosis and lymphocytopaenia in eight TBF-infected sheep

Sheep No.	Lymphocytosis (mm^2)	Lymphocytopaenia (mm^2)
291	69	4,344
292	26	2,843
294	67	1,819
295	60	3,738
297	101	2,895
299	311	1,869
319	187	3,098
320	287	3,308
Mean	138.5	2,989
Standard deviation	109.7	859

Table 3.6 Means and standard errors of differences from pre-inoculation counts of eosinophils and monocytes ($x10^9/l$) in eight TBF-infected sheep.

Days after inoculation	Parasitaemia	Eosinophil	Monocyte
0	-		
1	-	0.01 ± 0.02[1]	-0.01 ± 0.02[1]
2	-	0.02 ± 0.04	0.003 ± 0.03
3	+	0.1 ± 0.04	-0.02 ± 0.03
4	+	0.1 ± 0.04**	-0.1 ± 0.08
5	+	0.2 ± 0.03**	-0.2 ± 0.1
6	+	0.1 ± 0.04*	-0.1 ± 0.06
7	+	0.2 ± 0.05*	-0.05 ± 0.04
8	+	0.2 ± 0.05**	-0.03 ± 0.03
9	+	0.2 ± 0.04**	-0.02 ± 0.04
10	+	0.2 ± 0.05*	0.001 ± 0.04
11	-	0.2 ± 0.06[1]*	0.02 ± 0.02[1]
12	-	0.1 ± 0.05*	0.02 ± 0.03
13	-	0.1 ± 0.04[1]*	0.04 ± 0.02[1]
14	-	0.2 ± 0.04[1]**	0.04 ± 0.02[1]
15	-	0.1 ± 0.05*	0.03 ± 0.03
16	-	0.1 ± 0.05*	0.0 ± 0.04
17	-	0.1 ± 0.04*	-0.04 ± 0.04
18	-	0.1 ± 0.06	0.1 ± 0.1
19	-	0.1 ± 0.04	0.01 ± 0.3
20	-	0.01 ± 0.02[1]	0.01 ± 0.03[1]

* $P < 0.050$

** $P < 0.010$

[1]Derived from seven observations

Table 3.7 Number of days when no eosinophils were detected in eight TBF-infected sheep

Sheep No.	Days
291	7
292	5
294	1
295	5
297	3
299	4
319	5
320	5

Table 3.8 Magnitudes of the eosinopaenia and monocytosis in eight TBF-infected sheep

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
291	4,160	2,055
292	4,360	649
294	4,989	998
295	4,577	348
297	3,353	2,541
299	5,587	4,315
319	5,693	4,511
320	7,890	244
Mean	4,607.0	1,955.0
Standard deviation	787.4	1,717.8

Table 3.9 Maximal neutrophil counts and neutrophil count nadirs in eight TBF-infected sheep

Sheep No.	Maximal neutrophil count ($x10^9/l$)	Days after the onset of visible parasitaemia	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia
291	3.5	0	0.4	6
292	4.4	1	0.7	9
294	3.0	0	0.7	5
295	3.1	0	0.4	8
297	5.0	0	0.3	10
299	4.7	0	0.4	7
319	4.0	3	0.3	7
320	3.5	0	0.5	7
mean	3.9	0.5	0.4	7.3
standard deviation	0.7	1.0	0.1	1.6

Table 3.10 Magnitudes of the neutrophilia and neutropaenia in eight TBF-infected sheep

Sheep No.	Neutrophilia (mm^2)	Neutropaenia (mm^2)
291	395	3,556
292	200	2,881
294	82	3,199
295	0	5,572
297	521	4,151
299	459	3,443
319	1,182	2,514
320	761	3,472
Mean	450.0	3,598
Standard deviation	385.6	933

Table 3.11 Maximal parasitaemias and magnitude of the parasitaemias in eight TBF-infected sheep

Sheep No.	Maximal parasitaemia (log_{10} infected neutrophils/l)	Days after the onset of visible parasitaemia	Magnitude of parasitaemia
291	9.31	2	13,512
292	9.45	2	18,037
294	9.03	0	15,117
295	9.16	0	15,552
297	9.16	1	17,052
299	9.29	0	17,405
319	9.05	4	17,237
320	9.23	5	17,312
mean	9.20	1.7	16,403
standard deviation	0.1	1.9	1,528

Table 3.12 Significance of changes in the total peripheral blood lymphocyte counts in eight sheep before and after inoculation with *C. phagocytophila*

Days after inoculation	Mean and standard deviation of total lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation counts ($x10^9/l$)
0	14.4 ± 2.8	
7	7.4 ± 3.9	5.8 ± 1.3**
14	11.6 ± 2.2	2.8 ± 1.3
21	14.7 ± 1.4	-1.5 ± 1.8

** $P < 0.010$

Table 3.13 Significance of changes in the sIg^+ lymphocyte counts in eight sheep before and after inoculation with C. phagocytophila

Days after inoculation	Mean and standard deviation of sIg^+ lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation counts ($x10^9/l$)
0	1.6 ± 0.3	
7	0.6 ± 0.4	1.0 ± 0.2**
14	2.2. ± 0.7	-0.6 ± 0.2
21	3.4 ± 1.3	-1.8 ± 0.4**

* $P < 0.050$

** $P < 0.010$

Table 3.14 Mean percentages of sIg^+ and PNA^+ lymphocytes in the peripheral blood lymphocytes of eight sheep before and after inoculation with *C. phagocytophila*

Days after inoculation	Mean and standard deviation of total lymphocyte counts ($x10^9/l$)	Mean percentages and standard deviation of sIg^+ and PNA^+ lymphocytes	
		sIg^+	PNA^+
0	14.4 ± 3.8	11.9 ± 3.4	43.9 ± 9.1
7	7.4 ± 3.9	7.6 ± 6.3	46.5 ± 12.8
14	11.6 ± 2.2	19.5 ± 5.7	52.9 ± 7.1
21	14.7 ± 1.4	21.0 ± 4.8	47.5 ± 12.5

Table 3.15 Significance of changes in the PNA^+ lymphocyte counts in eight sheep before and after inoculation with C. phagocytophila

Days after inoculation	Mean and standard deviation of PNA^+ lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation counts ($x10^9/l$)
0	6.3 ± 2.1	
7	4.2 ± 2.5	2.0 ± 1.2
14	6.1 ± 1.1	0.2 ± 0.7
21	7.4 ± 1.7	-1.1 ± 0.7

Fig. 3.1 Mean febrile reaction in eight sheep infected with *C. phagocytophila*.

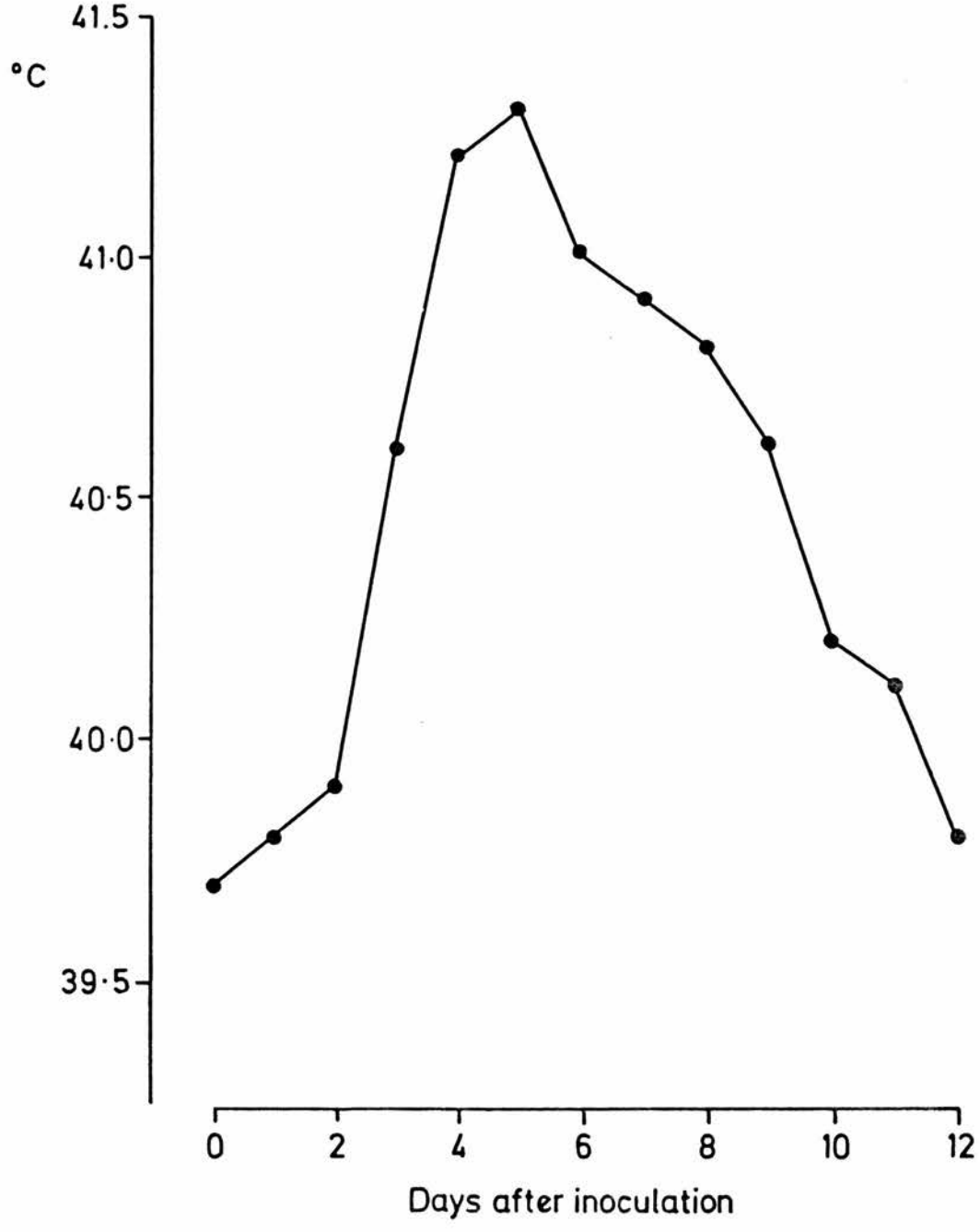

41.5
°C
41·0
40·5
40·0
39·5
0
2
4
6
8
10
12
Days after inoculation

Fig. 3.2 Mean daily total leucocyte, lymphocyte and neutrophil counts in eight sheep infected with *C. phagocytophila*.

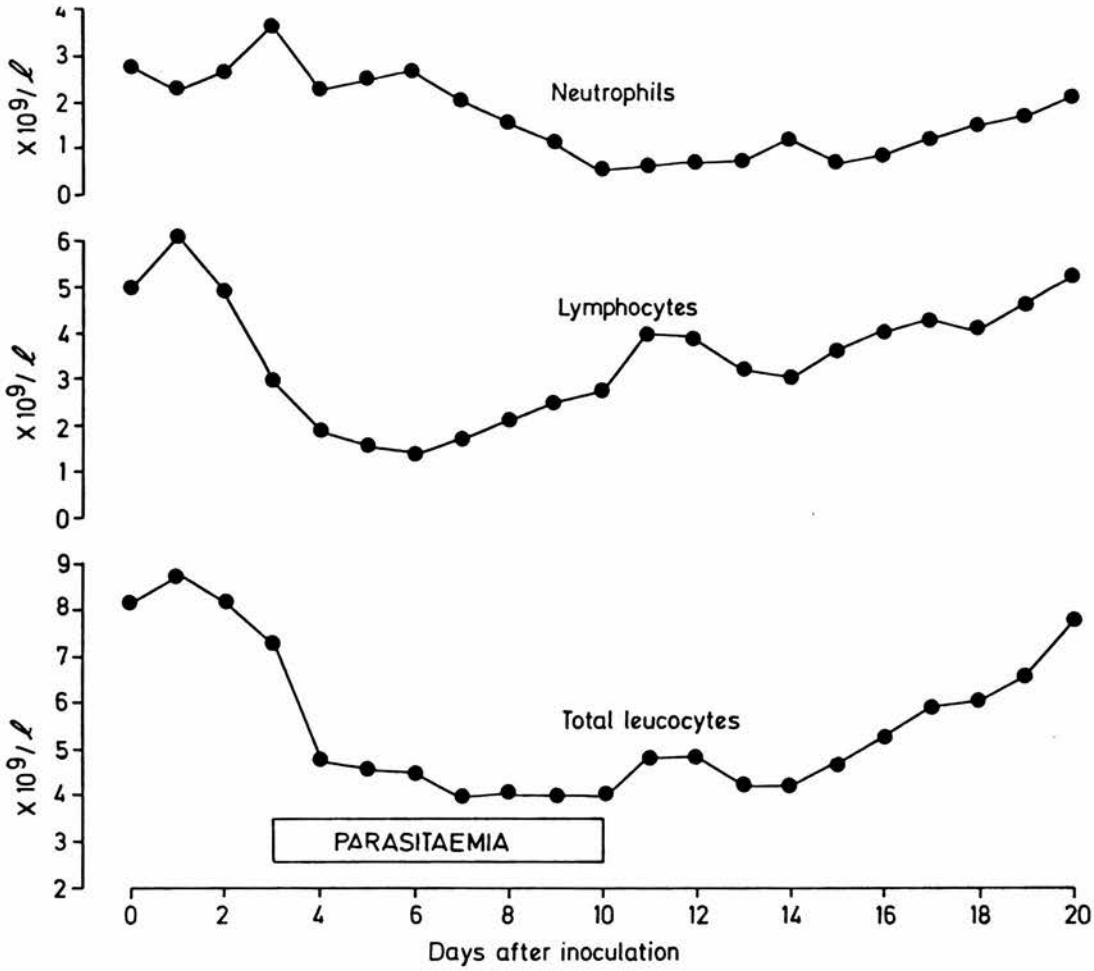

Neutrophils
Lymphocytes
Total leucocytes
PARASITAEMIA
X 10⁹/ℓ
Days after inoculation
0
2
4
6
8
10
12
14
16
18
20

Fig. 3.3 Medians of the daily eosinophil and monocyte counts in eight sheep infected with *C. phagocytophila*.

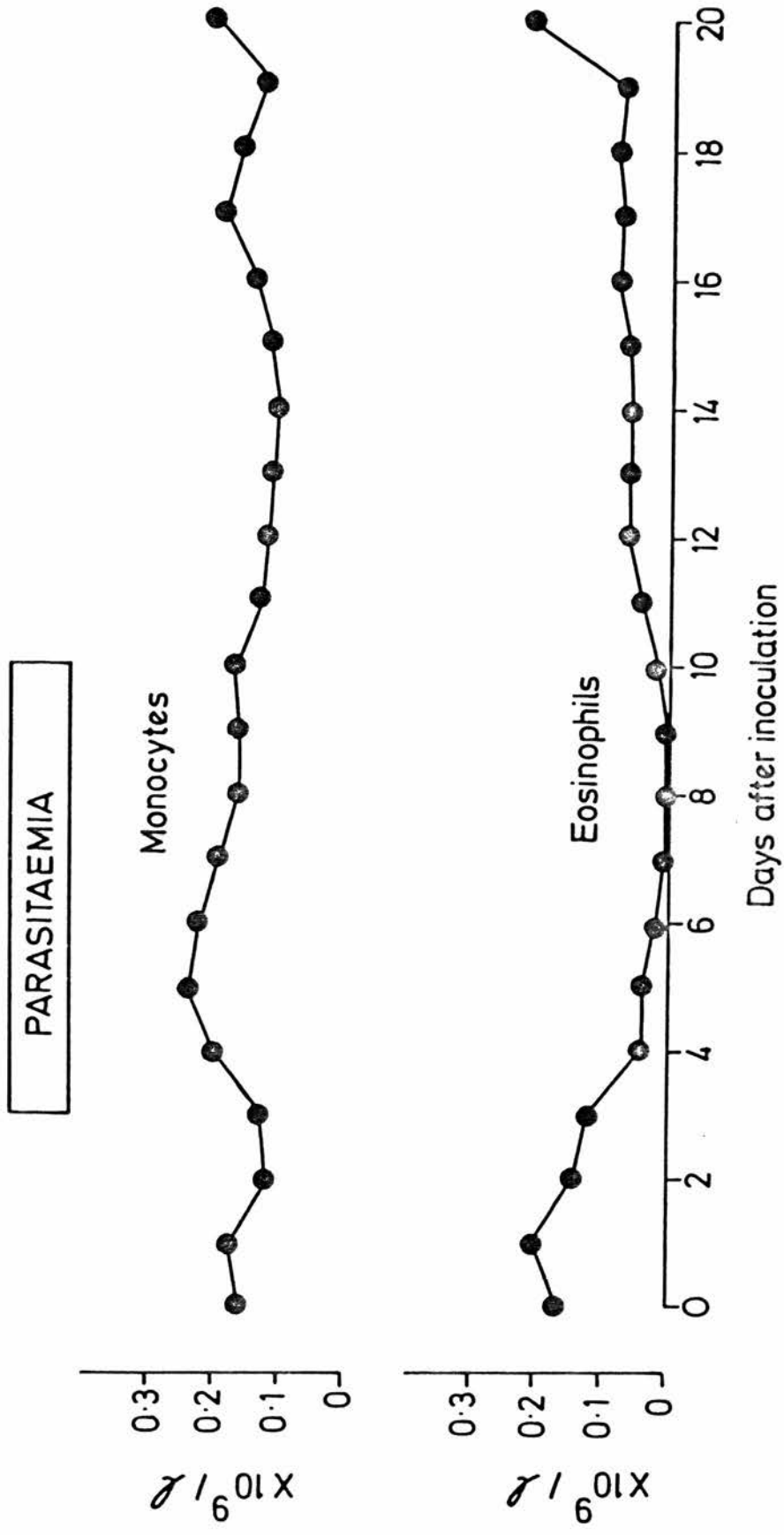
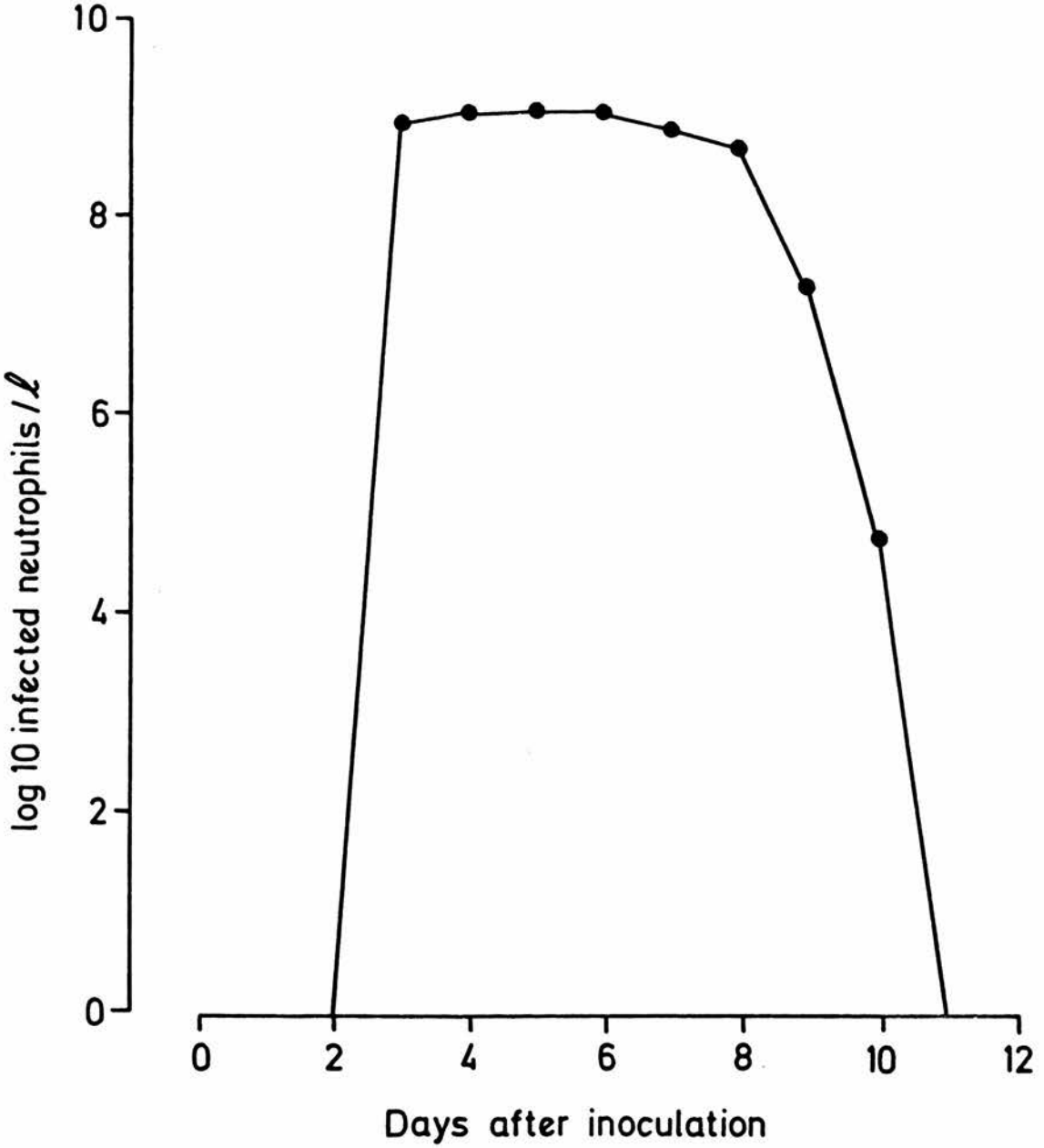

PARASITAEMIA
Monocytes
Eosinophils
x10⁹/ℓ
0·3
0·2
0·1
0
0
2
4
6
8
10
12
14
16
18
20
Days after inoculation

Fig. 3.4 Mean parasitaemic reaction in eight sheep infected with *C. phagocytophila*.


10
8
6
4
2
0
log 10 infected neutrophils / ℓ
0
2
4
6
8
10
12
Days after inoculation

CHAPTER FOUR

EFFECT OF TICK-BORNE FEVER ON IMMUNE FUNCTIONS IN SHEEP

Hudson in 1950 remarked that the effect of TBF on the leucocytes was such that the normal defence mechanisms were likely to be interfered with and, therefore, TBF could be expected to complicate other infections. Taylor and his colleagues (1941) had earlier suspected that the development of tick pyaemia was linked to a concurrent infection with TBF. Attention was then focussed on the neutropaenia as the predisposing factor; Foggie (1956), for example, showed that lambs suffering from a neutropaenia resulting from an attack of TBF were more susceptible to a blood-borne staphylococcal infection than normal lambs. The key role of the lymphocytes in defence had not been recognised at that time (Grabar, 1976).

The present study was designed to examine the effect of TBF on the humoral response to Clostridium chauvoei vaccine in sheep and to determine the effect of C. phagocytophila infection on cell-mediated immune response.

MATERIALS AND METHODS

Evaluation of Humoral Antibody Response to Clostridium chauvoei Vaccine

Vaccine. A commercial blackleg (Cl. chauvoei) vaccine[1] was used. It was a fluid vaccine containing formolised whole culture

[1]Blackleg vaccine (A.P.), Wellcome, Beckenham, Kent

of strains of Cl. chauvoei precipitated with potash alum. The recommended dose of one ml of the vaccine was employed to vaccinate each sheep by the subcutaneous route.

Vaccination and inoculation. Twenty adult sheep known to be susceptible to tick-borne fever and found to be free of Cl. chauvoei agglutinins were divided randomly into two groups of ten. One group was injected subcutaneously with one ml of the clostridial vaccine and served as the control. The other ten animals were inoculated intravenously with one ml of 10^{-1} dilution of blood containing the Old Sourhope strain of C. phagocytophila and injected subcutaneously with one ml of the vaccine. The animals in both groups were revaccinated subcutaneously with one ml of the clostridial vaccine 21 days after the initial vaccination.

Observations. The course of the tick-borne fever parasitaemia was monitored as described in Chapter Three. The animals were bled for sera immediately before and after vaccination at weekly intervals.

Serum antibody assay. The antigen used for the agglutination test was kindly provided by Dr. C.M. Thorley of the Wellcome Research Laboratories. It was a suspension of killed Cl. chauvoei in buffered saline containing 0.1 percent formalin adjusted to match opacity tube No. 8 of the Burroughs Wellcome Opacity Set.

Agglutination test. The agglutination test as adapted to the microtitre technique by Dr. P.D. Walker, Wellcome Research Laboratories (personal communication) was used. The sera were heat-inactivated at 56°C for 30 minutes and were initially diluted 1/10

with PBS. Two-fold serial dilutions of the serum were made in the wells of U-type microtitre plates[1] containing 0.025 ml of PBS. Equal volume of the antigen was added to each well. An antigen control and negative serum control wells were set up for each test. The plates were covered, gently agitated and incubated for one hour at 37°C. Evidence of agglutination was sought after 30 and 60 minutes of incubation. The agglutinated antigen tended to settle and the reaction was gauged by the amount of deposit in the bottom of the wells and by the clarity of the supernatant fluid. The end-point was taken as the highest dilution of the serum that produced complete agglutination of the antigen.

Delayed Skin Hypersensitivity Test

A delayed skin hypersensitivity reaction to a contact sensitising agent, 2,4-dinitrochlorobenzene[2] (DNCB) was employed to measure cell-mediated immune response *in vivo* (Turk and Waters, 1969).

Twelve adult sheep were divided randomly into three groups of four. The sheep in the first two groups were sensitised with DNCB; 50 µl of a two percent solution of DNCB in olive oil was painted evenly over a shaved area at the back of each sheep. Ten days after priming, two of the sheep in each group were inoculated with one ml of 10^{-1} dilution of TBF-infected blood (Old Sourhope strain). The other two sheep in each group were given one ml of normal sheep blood by the intravenous route and served as controls. At the onset of visible TBF parasitaemia, all sheep in the first group were

[1]Cooke Microtitre System, Sterilin Products Ltd., Teddington, Middlesex

[2]Phase Separations Ltd., Queensferry, Clywd

challenged with DNCB; 10 µl of a 0.2 percent solution of DNCB in olive oil was applied over a shaved area, approximately two centimetres in diameter, on the left side of the neck. The sheep in the second group were similarly challenged with DNCB four days after the onset of the reaction.

Two of the sheep in the third group were inoculated intravenously with one ml of 10^{-1} dilution of TBF-infected blood, whilst the two remaining sheep were each given one ml of normal sheep blood by the intravenous route. All animals were sensitised with 50 µl of a two percent solution of DNCB in olive oil seven days after inoculation and then challenged ten days after priming with 10 µl of a 0.2 percent solution of DNCB in olive oil.

The animals were examined six hours after challenge to rule out immediate hypersensitivity reactions. The DSH test was read 24, 48 and 72 hours after challenge by measuring the skin-fold thickness of the test and control sites using a dial micrometer. Changes in skin-fold thickness were estimated by subtracting the value for the control site from that of the test site.

RESULTS

Humoral Antibody Response to Clostridium chauvoei Vaccine

Primary response. The serum antibody titres to Cl. chauvoci vaccine in normal and TBF-infected sheep were expressed as the $\log_2$ reciprocals of the agglutination end-points. The mean titre in normal sheep seven days after vaccination was 4.7 ± 0.4 and this increased significantly to 5.3 ± 0.4 on day 14 ($t_9 = 2.713$, $P < 0.050$) (Table 4.1). The mean antibody titre continued to rise to 5.6 ± 0.5

on day 21 but the rise was not significant ($t_9 = 1.973$, $P > 0.050$) (Table 4.1).

The mean antibody titre in the infected sheep was 4.3 ± 0.5 on day seven and this was unchanged at 14 and 21 days after vaccination ($t_9 = 0.802$, $P > 0.50$ and $t_9 = 1.311$, $P > 0.50$, respectively) (Table 4.1).

The mean titres in the infected and normal sheep seven days after the initial vaccination were similar ($t_{18} = 2.469$, $P > 0.050$) (Table 4.1). However, significant differences in the mean antibody titres were evident on day 14 and 21 ($t_{18} = 3.538$, $P < 0.010$ and $t_{18} = 4.241$, $P < 0.001$, respectively) (Table 4.1).

Secondary response. A significant rise in the mean antibody titre was detected in the normal sheep after the second vaccine injection (Fig. 4.1). The rise however took three weeks to become evident (Table 4.2).

Likewise, a second antibody response was elicited in the infected sheep by the second dose of the vaccine (Fig.4.1). As before, the significant increase in response was delayed three weeks (Table 4.3).

Nevertheless, the mean antibody titres of the infected sheep after the second dose of the vaccine were all significantly lower than those of the normal sheep (Table 4.4, Fig. 4.1).

Delayed Skin Hypersensitivity Reaction

Measurements of the skin-fold thickness at 24, 48 and 72 hours after challenge with DNCB showed that the skin reaction was maximal

24 hours after challenge and then gradually subsided over the next 24 and 48 hours (Table 4.5). Tick-borne fever did not suppress the development of DSH reaction in sheep in groups one and two which were sensitised previously with DNCB.

The sheep in group three which were primed with DNCB during the reaction to C. phagocytophila infection developed contact sensitivity to DNCB and were able to mount a DSH reaction similar to that shown by the normal sheep when challenged later with DNCB (Table 4.5).

DISCUSSION

It is now accepted that immune responses are mediated by two major classes of lymphocytes: the B- and T-lymphocytes (Raff, 1973). Procedures for assessing B- and T-lymphocyte functions were developed recently and have been used in the investigation of abnormalities in the immune system. Response to immunisation, for example, has been employed for evaluating B-lymphocyte function; the presence of specific antibody in the serum being taken as evidence for the presence of B-lymphocytes which recognize antigen, co-operate with T-lymphocytes and macrophage and synthesize antibody (Perryman, 1979). The ability of lymphocytes to undergo blast transformation in vitro when stimulated with PHA or concanavalin A (Con A) has been utilised as a test of T-lymphocyte function (Janossy and Greaves, 1971). The DSH reaction employing DNCB for sensitisation and challenge has proved to be an accepted measure of in vivo T-cell activity or cell-mediated immune responsiveness to specific antigen (Turk and Waters, 1969; Hodgin, McGuire, Perryman and Grant, 1978). Rejection

of allogeneic skin grafts is also a well recognised *in vivo* procedure for evaluating T-lymphocyte function in animals (Dennis, Jacoby and Griesemer, 1969).

Studies on immunosuppression due to microbial infections have been carried out with leukaemia viruses and certain non-oncogenic viruses which infect or replicate in lymphoid tissues. Infection of mice with Friend virus (Wedderburn and Salaman, 1968), Rauscher leukaemia virus (Siegel and Morton, 1966), and Maloney leukaemia virus (Cremer *et al.*, 1966), for example, have been reported to depress the primary and secondary antibody responses to SRBC. Mims and Wainwright (1968) found that mice infected with the non-leukaemic virus, LCM virus, had fewer antibody-forming cells and produced four- to eight-fold less antibody to SRBC than the non-infected mice, and also had reduced capacity to react with footpad swelling against other viruses. It was postulated that the immunosuppression associated with LCM virus infection is due to a depletion of the number of immunocompetent cells rather than a reduced reactivity of cells involved in the immune response (Mims and Wainwright, 1968; Brø-Jorgensen and Volkert, 1974).

Immunosuppression has been observed in ruminants infected with trypanosomes where antibody responses to polyvalent clostridial vaccine (Holmes *et al.*, 1974). *Vibrio foetus* antigen (Mackenzie *et al.*, 1975), foot-and-mouth disease vaccine (Scott *et al.*, 1977) and louping-ill vaccine (Whitelaw *et al.*, 1979) were suppressed following or during acute infections. Urquhart and his colleagues (1973) noted that when *Nippostrongylus brasiliensis* infection was superimposed on a pre-existing *Trypanosoma brucei* infection in rats,

the normal process of immune expulsion of adult worms did not occur and the production of circulating protective antibody (IgG) and of IgE was considerably impaired whereas, cell-mediated immunity as measured by DSH reaction to oxazolone was unaffected.

Murray and his co-workers (1974) observed that B-cell function was defective during the acute phase of T. brucei infection but in more long-standing infections both T- and B-cell functions were affected. B-memory cells for thymus-dependent and thymus independent antigens were either depleted or had lost their potential to respond to antigens during T. brucei infection (Askonas, Corsini, Clayton and Ogilvie, 1979). The ability of T-cells to respond to PHA and Con A were diminished (Corsini et al., 1977; Jayawardena and Waksman, 1979), T-helper cells and cells reactive to allogeneic target cells in mixed lymphocyte reactions were found to be functionally defective but the cells affecting delayed hypersensitivity reactions retained their activity throughout the course of the infection (Askonas et al., 1979). Clayton, Ogilvie and Askonas (1979) showed that functional T-lymphocytes were not necessary for trypanosome mitogenecity or immunosuppression as both phenomena were readily demonstrable in athymic mice. They hypothesised that a mitogenic trypanosome component while by aspecifically stimulating the multiplication of B-cells, prevented their subsequent participation in the immune responses.

Reduced primary and secondary responses to sheep erythrocytes and human gamma-globulin have been observed to occur in mice infected with Plasmodium berghei (Greenwood et al., 1971). Malarial infection in man was reported to cause diminished antibody titres

to tetanus toxoid, and children with acute malaria were found to have decreased antibody responses to 'O' but not to 'H' antigen of Salmonella typhii (McGregor and Barr, 1962; Greenwood et al., 1972). The T-cell functions appeared not to be affected as P. berghei yoelli infection in mice did not affect skin graft rejection or contact hypersensitivity (Greenwood et al., 1971). It was suggested that immunosuppression in malaria is due to a disturbance in macrophage function induced by the infection or to an impairment in macrophage antigen processing (Loose et al., 1973; Tanabe et al., 1977).

The effect of TBF on the humoral immune response to Cl. chauvoei vaccine in sheep was as expected; injections of the vaccine induced primary and secondary antibody responses in the normal and TBF-infected sheep. Serum agglutinating antibodies were detected seven days after the initial vaccination. In the normal sheep, the mean antibody titre increased significantly by day 14 and continued to rise whereas, in the infected sheep the mean antibody titre was unchanged at 14 and 21 days after vaccination. The differences in the antibody titres of the two groups were significant at 14 and 21 days but not at seven days. The mean antibody titres of the normal sheep after the second dose of the vaccine were consistently and significantly higher than the mean titres of the TBF-infected sheep.

A significant increase in the mean antibody titre was detected in the normal and in the infected sheep after the second dose of the vaccine despite the fact that the antibodies being measured were

agglutinating antibodies and, therefore, were likely to be associated with the IgM serum fraction rather than the IgG fraction. It has been recognised that injection of antigen in adequate amounts resulted initially to the production of high molecular weight (19S) antibody of the IgM class, to be superseded in the majority of instances by the synthesis of 7S antibody of the IgG class; the antibody formed in the secondary response to antigen being IgG although under certain conditions IgM can also be detected (Bauer and Stavitsky, 1961; Nossal, Austin and Ada, 1965; Uhr and Finkelstein, 1967). In a study of the secondary activities of highly purified rabbit gamma-M and gamma-G antibodies to the somatic antigens of Salmonella typhimurium, Robbins and his colleagues (1965) demonstrated that the gamma-M class of immunoglobulin was 22 times as active as the gamma-G immunoglobulin in inducing agglutination of particulate antigens.

In contrast to the suppressed humoral immune response, cell-mediated immune response as measured by a DSH reaction to DNCB was, as expected, not affected by TBF. The sheep that were primed with DNCB during the reaction to C. phagocytophila infection developed contact sensitivity to DNCB and were able to mount a DSH reaction similar to that of the normal sheep when challenged later with DNCB. Tick-borne fever also did not inhibit the development of a DSH reaction in sheep previously sensitised with DNCB.

Challenge infection of the vaccinated sheep with Cl. chauvoei was not performed in the present study, hence, the effect of TBF on the level or development of vaccine-induced protection was not adequately assessed. Nevertheless, the considerable reduction in

humoral response to the vaccine that was found to occur during the reaction to TBF confirms the immunosuppressive effect of C. phagocytophila in sheep.

Table 4.1 Means and standard deviations of the serum agglutinating antibody titres to Cl. chauvoei vaccine in groups of ten normal and ten TBF-infected sheep.

Days after initial vaccination	Normal sheep	Infected sheep
7	4.7 ± 0.4	4.3 ± 0.5
14	5.3 ± 0.4	4.5 ± 0.5
21	5.6 ± 0.5	4.1 ± 1.0

Table 4.2 Mean serum agglutinating antibody titres in ten normal sheep before and after the second dose of Cl. chauvoei vaccine

Days after second vaccination	Antibody titre ($\log_2$ reciprocal ± sd)	Difference from titre recorded before second vaccination	$t_{(9)}$
0	5.6 ± 0.5		
7	5.8 ± 0.9	-0.2	0.613
14	5.9 ± 0.3	-0.3	1.973
21	6.5 ± 0.5	-0.9	3.862**
28	6.2 ± 0.4	-0.6	2.715*
35	6.2 ± 0.4	-0.6	2.715*

* $P < 0.050$

** $P < 0.010$

Table 4.3 Mean serum agglutinating antibody titres in ten TBF-infected sheep before and after the second dose of *Cl. chauvoei* vaccine

Days after second vaccination	Antibody titre ($\log_2$ reciprocal ± sd)	Difference from titre recorded before second vaccination	$^{t}(9)$
0	4.1 ± 1.0		
7	4.1 ± 0.5	0	0
14	4.6 ± 0.7	-0.5	1.102
21	5.6 ± 0.7	-1.5	3.144*
28	5.3 ± 0.6	-1.2	3.675**
35	5.0 ± 0.4	-0.9	3.251**

* $P < 0.020$

** $P < 0.010$

Table 4.4 Means and standard deviations of the serum agglutinating antibody titres to Cl. chauvoei vaccine in groups of ten normal and ten TBF-infected sheep

Days after second vaccination	Normal sheep	Infected sheep	$t_{(18)}$
7	5.8 ± 0.9	4.1 ± 0.5	4.967***
14	5.9 ± 0.3	4.6 ± 0.7	5.405***
21	6.5 ± 0.5	5.6 ± 0.7	3.251**
28	6.2 ± 0.4	5.3 ± 0.6	3.599**
35	6.2 ± 0.4	5.0 ± 0.4	6.020***

**P < 0.010

***P < 0.001

Table 4.5 Delayed skin hypersensitivity reactions: average increase in skin-fold thickness (mm) of two normal and two TBF-infected sheep

Hours after challenge with DNCB	EXPERIMENTAL GROUP					
	Normal	Infected[1]	Normal	Infected[2]	Normal	Infected[3]
24	6.60	6.35	7.11	5.08	6.85	5.84
48	5.58	5.33	5.08	4.57	5.08	4.82
72	5.08	4.57	4.06	3.18	4.06	4.06

1 = sensitised with DNCB, then infected ten days later with C. phagocytophila and challenged with DNCB at the onset of TBF parasitaemia.

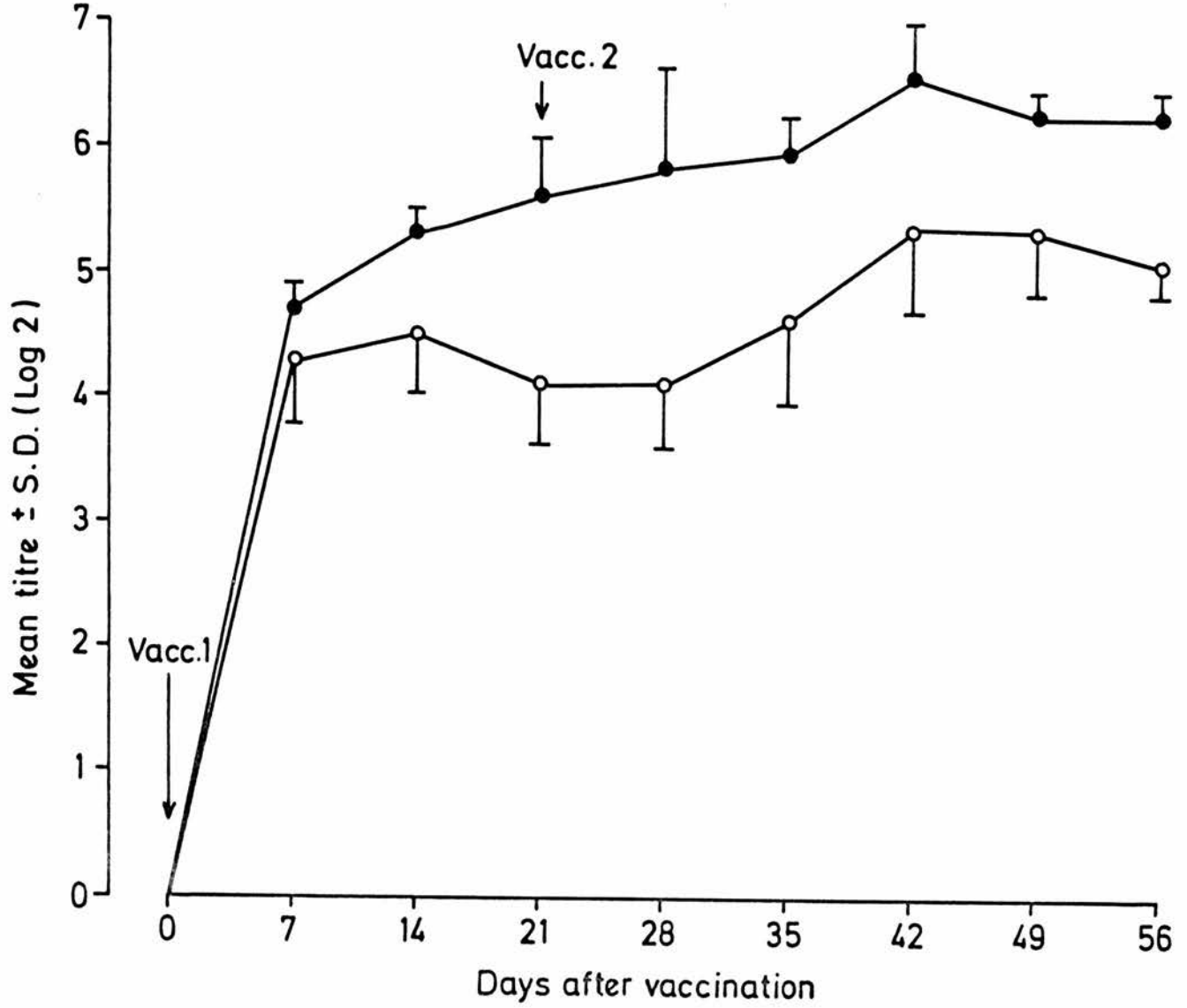
2 = sensitised with DNCB, then infected ten days later with C. phagocytophila and challenged with DNCB four days after the onset of TBF parasitaemia.

3 = infected with C. phagocytophila, then sensitised with DNCB four days after the onset of TBF parasitaemia and challenged ten days later with DNCB.

Fig. 4.1 Mean serum agglutinating antibody titres to Cl. chauvoei vaccine in groups of ten normal and ten TBF-infected sheep.

● Normal sheep

o TBF-infected sheep


Vacc. 2
Vacc.1
Mean titre ± S.D. (Log 2)
0
1
2
3
4
5
6
7
0
7
14
21
28
35
42
49
56
Days after vaccination

CHAPTER FIVE

EFFECT OF TICK-BORNE FEVER ON PHAGOCYTOSIS BY NEUTROPHIL LEUCOCYTES

Taylor, Holman and Gordon in 1941 studied the haematological responses of sheep infected with C. phagocytophila and found that neutropaenia was a distinguishing feature of TBF. Subsequently, Foggie (1957) investigated the effect of TBF on the susceptibility of lambs to staphylococcal infections. He showed by using blood from a normal lamb and a TBF-infected lamb that the bacteriostatic effect of blood on the staphylococci was reduced during the TBF neutropaenia. Foster and Cameron (1970a) demonstrated by an in vivo test that diapedesis of neutrophils containing TBF-inclusion bodies was inhibited and they postulated that the neutropaenia of TBF was perhaps preceded by a degree of functional impairment of the neutrophils.

The present study was designed to extend these earlier investigations on the effect of TBF on phagocytosis by using larger numbers of sheep and more refined techniques.

MATERIALS AND METHODS

Animals

These studies were carried out concurrently with sheep used to assay the clinical, haematological and parasitological parameters of TBF as recorded in Chapter Three. Blood samples were collected from the jugular vein into 20 ml evacuated tubes[1]

[1]Vacutainer, Becton-Dickinson and Co., Rutherford, New Jersey

containing preservative-free heparin[1] (10 units/ml) immediately before and then three days after the onset of the visible parasitaemia.

Preparation of polymorphonuclear leucocyte suspension

The polymorphonuclear (PMN) leucocytes were isolated from whole blood according to the method described by Phillips and his colleagues (1979). The heparinised blood was diluted with an equal volume of sterile HBSS. Four ml of the diluted blood were layered over three ml of Ficoll-Paque in a centrifuge tube and then spun at 400 g for 35 minutes. The layers containing the plasma, platelets, mononuclear cells and Ficoll-Paque were discarded, leaving the bottom layer of erythrocytes and PMN leucocytes. The bottom layer was mixed with five ml of six percent Dextran[2] and 15 ml of sterile PBS. The erythrocytes were allowed to settle at room temperature for one hour. The PMN-rich supernatant fluid was collected and centrifuged at 700 g for five minutes. The deposit was washed three times with sterile heparin-saline (50 units heparin/100 ml saline) solution. The cells were suspended in buffered Hanks' solution, checked for viability by the trypan blue dye exclusion test, enumerated in a Coulter counter and made up to a concentration containing approximately 2-3 x 10^7 cells per ml in buffered Hanks' solution containing 0.1 percent gelatin[3].

[1]Pularin, Duncan, Flockhart and Co. Ltd., London

[2]Pharmacia, Fine Chemicals AB, Uppsala, Sweden

[3]Difco Laboratories, Detroit, Michigan

Preparation of bacterial cell suspension

A coagulase-positive strain of Staphylococcus pyogenes isolated from a local lamb was used. The bacteria were grown and maintained in nutrient agar slope in screw-capped bottles. For each phagocytosis experiment, a single colony of the bacteria was grown in 100 ml of a 2.5 percent nutrient broth for 18 hours at 37°C. Under these conditions, the growth pattern of the bacteria was found to be consistent, and after repeated determinations, the 18-hour broth culture when diluted 1:10 was found to contain approximately 2-3 x 10^7 cells per ml. The concentration of the bacterial suspension was also checked by turbidity measurements using the Burroughs Wellcome Opacity Set. The 18-hour broth culture was centrifuged for ten minutes at 1,500 g, washed three times in sterile normal saline and finally the pellet was suspended in gelatin-Hanks' solution to a concentration containing approximately 2-3 x 10^7 cells per ml. Before use, the clumps in the bacterial suspension were broken down thoroughly with a mechanical mixer[1].

Serum

Serum was collected from four lambs which had had naturally acquired staphylococcal infections. The sera were pooled and stored at -20°C.

Assay of phagocytosis

The in vitro assay for phagocytosis of staphylococci by neutrophil leucocytes was carried out according to the method described

[1]Griffin and George Ltd., Wembley, Middlesex

by Van Furth, Van Zwet and Leijh (1978). Two-ml volumes of PMN leucocytes and bacterial suspensions were combined with 0.4 ml of sheep serum in centrifuge tubes. A PMN leucocyte control tube containing two ml of PMN cell and bacterial suspensions and 0.4 ml of gelatin-Hanks' solution, and a bacterial control tube containing two ml of the bacterial suspension and 2.4 ml of gelatin-Hanks' solution were prepared at the same time. The tubes were tightly capped, incubated at 37°C in a water-bath and tumbled over every minute during incubation. Before and after 30, 60 and 120 minutes of incubation, an aliquot of 0.5 ml was taken from each mixture and added to 1.5 ml of ice-cold gelatin-Hanks' solution to stop phagocytosis. The samples were then centrifuged at 110 g for four minutes to sediment the PMN leucocytes. The supernatant fluid containing the non-ingested bacteria was removed and serial ten-fold dilutions in sterile PBS were made. Aliquots of 0.1 ml of the two highest dilutions were inoculated onto each of two DST agar plates[1] and incubated at 37°C for 24 hours. The colonies were counted and the number of viable bacteria per ml was calculated from the means of the colony counts of duplicate plates.

The PMN-cell pellet was washed three times in sterile normal saline-heparin solution and cell smears were prepared, fixed in methanol and stained with Giemsa. The slides were examined by light microscopy using an oil immersion objective. Phagocytosis was assessed by counting the number of neutrophils out of 100 PMN neutrophils that appeared to have ingested four or more bacteria.

[1]Diagnostic Sensitivity Medium Agar, Oxoid Ltd., London

This method of evaluating phagocytic activity was claimed to yield data that were more reproducible than the more commonly used determination of the number of bacteria ingested by 100 or more phagocytic cells (Klostergaard, Lisafeld, Dunlap, Klein and Holtermann, 1978).

Intracellular killing

The method described by Van Furth and his co-workers (1978) was employed in assessing intracellular killing of staphylococci by neutrophil leucocytes. Equal volumes, 1.5 ml, of the PMN-cell and bacterial suspensions were added to 0.4 ml of serum in a centrifuge tube. Control tube containing 1.5 ml of PMN-cell suspension and equal volume of the bacterial suspension and 0.4 ml of gelatin-Hanks' solution was prepared at the same time. The mixtures were incubated in a water-bath at 37°C and gently agitated during incubation. After 15 minutes of incubation, phagocytosis was stopped instantly by placing the tubes in crushed ice for one minute. The PMN cells were sedimented by centrifuging at 110 g for four minutes. The supernatant fluid containing the non-ingested bacteria was removed and the PMN-cell pellet was washed three times with gelatin-Hanks' solution to remove extracellular bacteria. The washed PMN leucocytes were resuspended in three ml of buffered Hanks' solution and reincubated in a water-bath at 37°C. At 0, 30, 60 and 120 minutes, a 0.5 ml sample of the cell suspension was taken and added to 0.5 ml of ice-cold buffered Hanks' solution to stop the intracellular killing of ingested bacteria. The sample was then centrifuged for four minutes at 110 g. The supernatant

fluid was removed and the PMN leucocytes were lysed by adding one ml of ice-cold distilled water with 0.1 percent of bovine serum albumin and by pipetting for 30 seconds. Serial ten-fold dilutions of each sample were made in sterile PBS and 0.1 ml aliquots of the two highest dilutions were inoculated onto each of two DST agar plates and incubated at 37°C for 24 hours. The colonies were counted and the number of viable bacteria remaining within the cells was estimated from the means of the colony counts of duplicate plates.

Calculations

Phagocytosis and intracellular killing of staphylococci by neutrophil leucocytes were measured *in vitro* over a given period of time and were expressed as the percent decrease of the initial number of viable bacteria. The phagocytic index was calculated as follows:-

$$F(t) = \frac{(N_o - N_t)}{N_o} \quad x\ 100$$

where $F(t)$ was the phagocytic index after t minutes, N_o was the initial number of viable bacteria and N_t was the number of viable extracellular bacteria after t minutes (Van Furth *et al.*, 1978). The intracellular killing index was calculated as follows:-

$$K(t) = \frac{(N_o - N_t)}{N_o} \quad x\ 100$$

where $K(t)$ was the intracellular killing index after t minutes, N_o was the initial number of viable intracellular bacteria and N_t was the number of viable intracellular bacteria after t minutes (Van Furth *et al.*, 1978).

RESULTS

The result of the phagocytic assays showed significant reductions in the indices during the reaction to C. phagocytophila infection even within 30 minutes' incubation (Table 5.1, Fig. 5.1).

Morphological assessment of phagocytosis on stained smears revealed that before inoculation, the mean percentage of neutrophils that had ingested one to three bacteria after 60 minutes of incubation was 71.1 $\pm$ 5.2 percent and 28.8 $\pm$ 5.2 percent had phagocytosed four or more bacteria (Table 5.2). During the reaction to TBF the number of non-infected neutrophils was found to be on the average 68.7 $\pm$ 6.1 cells per 100 neutrophils (Table 5.2). The phagocytic activity of the non-infected neutrophils was unaffected during the TBF parasitaemia, the mean percentage of non-infected neutrophils that had ingested one to three bacteria after 60 minutes of incubation being 74.3 $\pm$ 5.4; the mean percentage of non-infected neutrophils found engaged in active phagocytosis, i.e. containing four or more bacteria was 25.6 $\pm$ 5.4 percent (Table 5.2). In contrast, 76 $\pm$ 3.7 percent of the neutrophils containing TBF-inclusion bodies had not ingested bacteria after 60 minutes of incubation and the infected neutrophils that had phagocytosed contained no more than two bacteria, indicating that phagocytosis during the reaction to TBF was achieved mainly by the non-infected neutrophils (Table 5.3).

Sheep with higher parasitaemias had lower phagocytic indices. The negative correlation was significant ($r_6 = -0.76$, $P < 0.050$) (Table 5.4).

There was only a minimal reduction in the intracellular killing of staphylococci by neutrophil leucocytes during the TBF parasitaemia (Table 5.5, Fig. 5.2).

DISCUSSION

Phagocytosis is a fundamental function of neutrophil leucocytes and is one of the important innate defence mechanisms of the body against invading organisms (Suter, 1956). Measurement of this function, therefore, has been used to assess susceptibility to diseases (Ammann and Fudenberg, 1976; Newbould, 1976). Defects in the neutrophil function may be quantitative where the total number of functionally normal neutrophils is reduced below critical level allowing infection to ensue, or may be qualitative where the total number of circulating neutrophils is normal or even elevated but the cells fail to perform their normal microbicidal function (Stites, 1976).

In TBF, the total number of circulating neutrophils is markedly reduced during the reaction, and the bacteriostatic effect of TBF-infected blood on staphylococci as measured by an *in vitro* test was found to be significantly depressed during the neutropaenia (Foggie, 1957). Results of a subsequent study assessing neutrophil movement by the Rebuck skin window test, an *in vivo* technique, suggested impairment of diapedesis of neutrophils containing TBF-inclusion bodies (Foster and Cameron, 1970a).

In the present study, phagocytosis and intracellular killing of staphylococci by neutrophil leucocytes in sheep before and during the reaction to TBF were determined by *in vitro* techniques which allowed for the process of phagocytosis and intracellular killing of ingested bacteria to be assessed independently of each other. A constant number of PMN leucocytes was used throughout to exclude

the effect of neutropaenia on phagocytosis when whole blood from TBF-infected animals was used (Foggie, 1957). The results of the assays showed that phagocytosis of staphylococci by neutrophil leucocytes was reduced during TBF parasitaemia; this reduction was attributable to the limited phagocytic activity of neutrophils containing TBF-inclusion bodies, a functional impairment such as that postulated by Foster and Cameron (1970a). Morphological assessment of phagocytosis on stained smears of PMN leucocytes after 60 minutes of incubation with staphylococci revealed that while phagocytosis by non-infected neutrophils was not affected during the TBF parasitaemia, the phagocytic activity of the parasitised neutrophils was reduced considerably; 76 percent of the neutrophils containing TBF-inclusion bodies had not engaged in phagocytosis even after 60 minutes' incubation and those that had phagocytosed contained only from one to two bacteria per cell.

The intracellular killing of ingested organisms is the final stage of phagocytosis and is dependent on the completion of preceding stages: motility, recognition, ingestion and degranulation; a defect in intracellular killing follows failure of any one or combination of these functions (Stites, 1976). In the present study, the intracellular bactericidal activity of the neutrophil leucocytes was only slightly reduced during the reaction to TBF; the difference in the intracellular killing indices before inoculation and during the parasitaemia was not significant. The lack of difference in the efficiency of intracellular killing can be explained by the finding that phagocytosis in TBF-infected blood was

achieved mainly by non-parasitised neutrophil leucocytes and the presence of any defect in intracellular killing by parasitised neutrophils was therefore not readily apparent.

In summary TBF is accompanied by quantitative as well as qualitative changes in the neutrophil leucocytes and the degree of impairment of the neutrophil function depends not only on the magnitude of the neutropaenia but also on the number of TBF-infected neutrophils.

Table 5.1 Phagocytosis of staphylococci by neutrophil leucocytes in eight TBF-infected sheep before inoculation and during the reaction to C. phagocytophila infection; mean phagocytic index and standard deviation

Time (minutes)	Before inoculation	During the parasitaemia	$t_{(7)}$
30	52.6 ± 5.2	40.8 ± 7.1	5.223**
60	90.0 ± 2.2	77.0 ± 5.7	7.692***
120	95.1 ± 3.1	88.2 ± 2.6	8.192***

** $P < 0.010$

*** $P < 0.001$

Table 5.2 Morphological assessment of phagocytosis by neutrophils after 60 minutes' incubation with staphylococci before inoculation and during the reaction to C. phagocytophila infection

Sheep No.	Before inoculation		During the parasitaemia		
	Percentage with 1 to 3 bacteria	Percentage with 4 or more bacteria	Number of non-infected neutrophils per 100 neutrophils	Percentage non-infected neutrophils with 1 to 3 bacteria	Percentage non-infected neutrophils with 4 or more bacteria
291	72	28	64	80	20
292	80	20	59	75	25
294	77	23	72	81	19
295	67	33	64	80	20
297	64	36	74	67	33
299	68	32	67	70	30
319	70	30	74	72	28
320	71	29	76	70	30
Mean	71.1	28.8	68.7	74.3	25.6
Standard deviation	5.2	5.2	6.1	5.4	5.4

Table 5.3 Morphological assessment of phagocytosis by neutrophils after 60 minutes' incubation with staphylococci during the reaction to *C. phagocytophila* infection

Sheep No.	Number of infected neutrophils per 100 neutrophils	Infected neutrophils with no bacteria		Infected neutrophils with one to two bacteria	
		Number	Percentage	Number	Percentage
291	36	29	80.5	7	19.4
292	41	30	73.0	11	26.8
294	28	22	78.5	6	21.4
295	36	27	75.0	9	25.0
297	26	20	77.0	6	23.0
299	33	25	75.7	8	24.0
319	26	18	69.0	8	30.7
320	24	19	79.0	5	20.8
Mean	31.2	23.7	76	7.5	23.8
Standard deviation	6.1	4.6	3.7	1.9	3.6

Table 5.4 Relationship of visible parasitaemia to Phagocytic index

Sheep No.	Parasitaemia (log_{10} infected neutrophils/l)	Phagocytic index
291	9.16	85
292	9.30	87
294	8.82	91
295	8.93	86
297	9.02	87
299	9.05	87
319	8.94	91
320	8.80	92

Table 5.5 Intracellular killing of S. pyogenes by neutrophil leucocytes in eight sheep before inoculation and during the reaction to C. phagocytophila infection; mean intracellular killing index and standard deviation

Time (minutes)	Before inoculation	During TBF parasitaemia	Mean and standard error of difference from pre-inoculation intracellular killing index	$t_{(7)}$
30	61.3 ± 6.0	55.0 ± 4.3	6.8 ± 5.8	1.172
60	86.5 ± 2.7	82.1 ± 6.0	4.3 ± 4.2	1.023
120	93.2 ± 1.4	89.6 ± 4.4	3.6 ± 1.7	2.117

Fig. 5.1 *In vitro* phagocytosis of staphylococci by neutrophil leucocytes in eight sheep before inoculation and during the reaction to *C. phagocytophila* infection.

- ● Before inoculation
- o During reaction to TBF

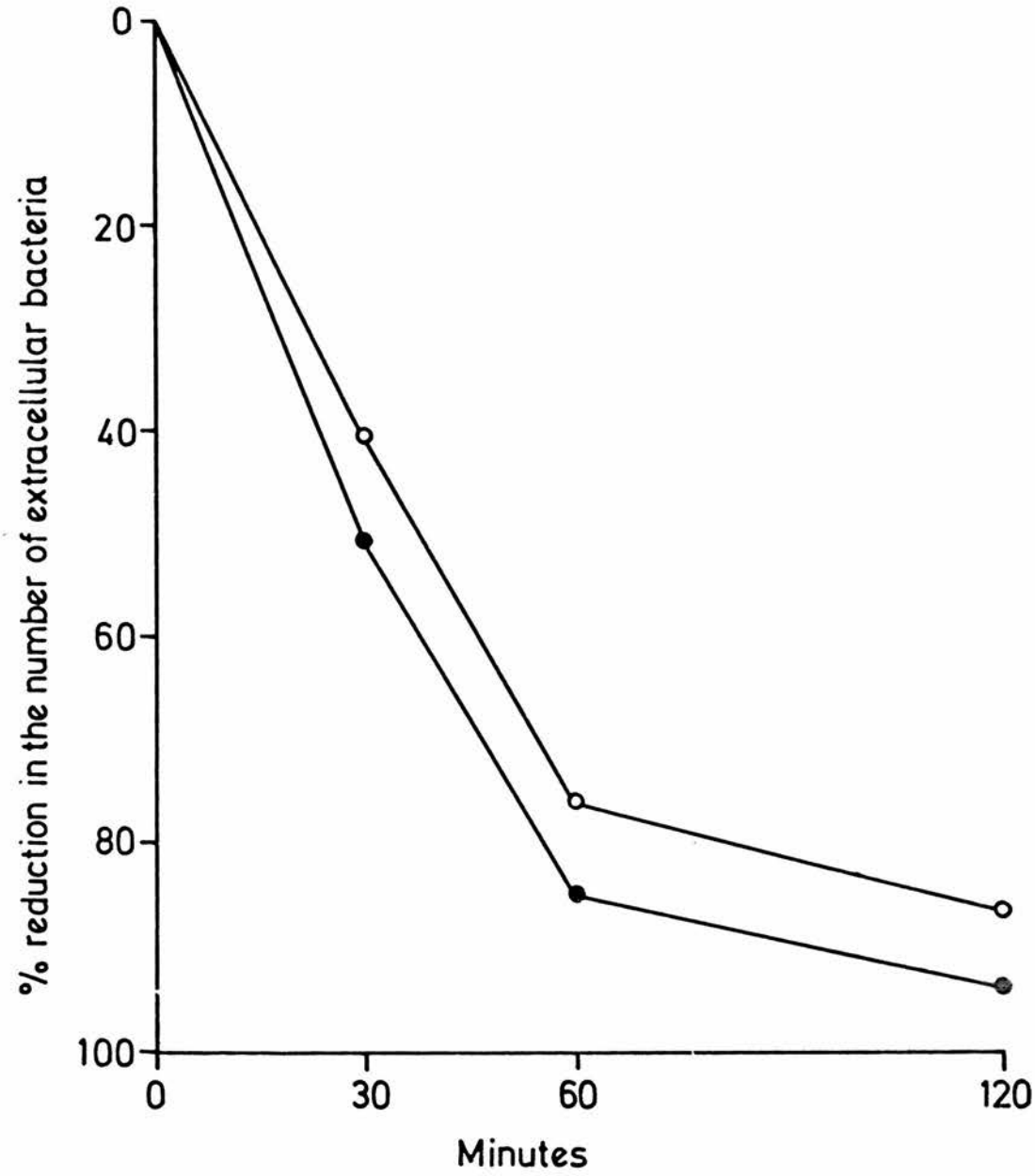
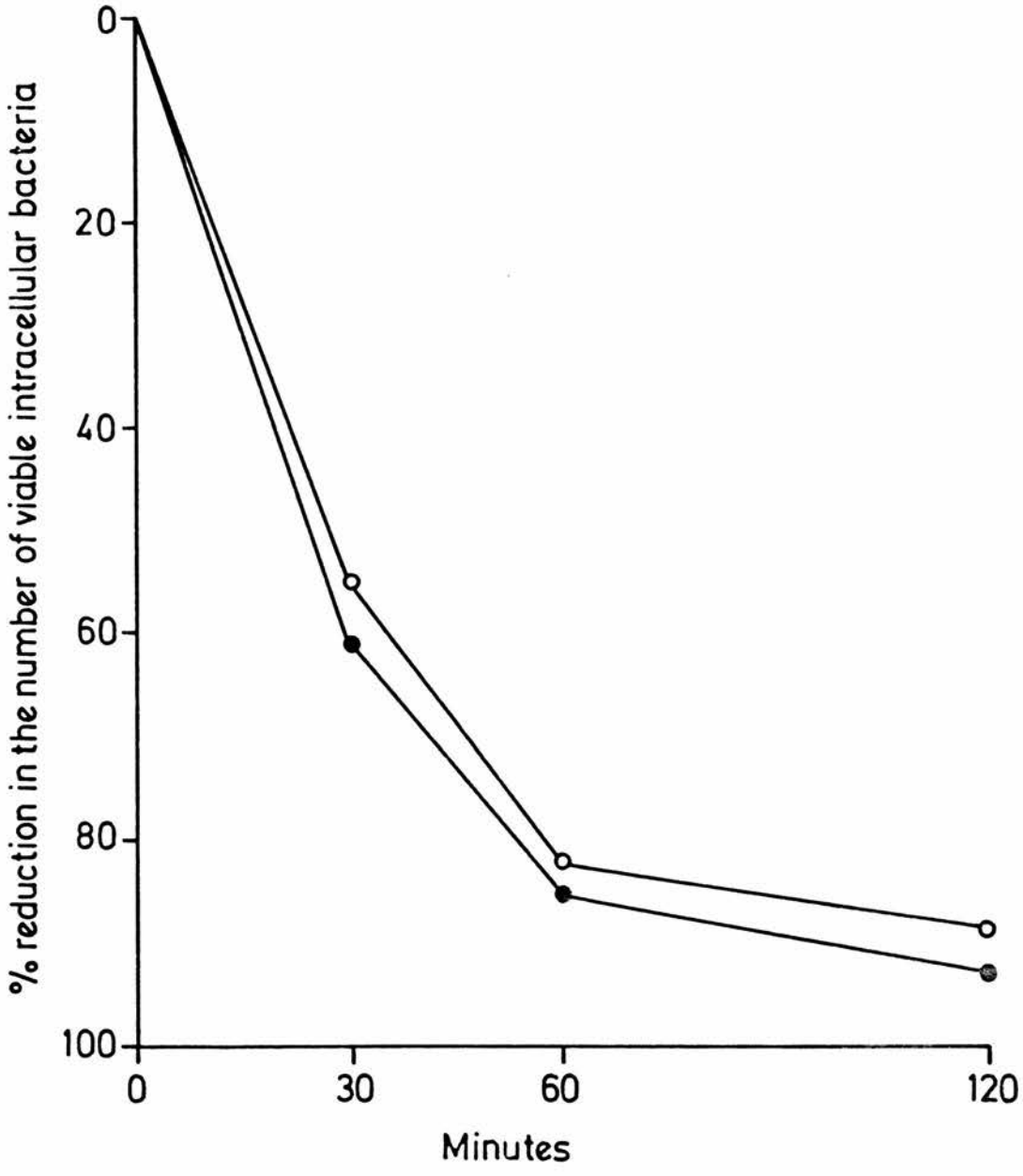

0
20
40
60
80
100
% reduction in the number of extracellular bacteria
0
30
60
120
Minutes

Fig. 5.2 Intracellular killing of staphylococci by neutrophil leucocytes in eight sheep before inoculation and during the reaction to *C. phagocytophila* infection

● Before inoculation

○ During reaction to TBF


0
20
40
60
80
100
% reduction in the number of viable intracellular bacteria
0
30
60
120
Minutes

CHAPTER SIX

NASAL CARRIAGE OF PASTEURELLA HAEMOLYTICA IN SHEEP INFECTED WITH TICK-BORNE FEVER

Pasteurella haemolytica is an opportunist pathogen often isolated from the upper respiratory tract of apparently normal sheep (Gilmour, I978). It is commonly associated with respiratory disease in sheep but there has been considerable doubt whether this organism is in fact capable of causing disease (Stamp, Watt and Thomlinson, 1955). Attempts to produce disease with P. haemolytica alone have met with variable success leading to the suggestion that unidentified predisposing factors are involved (Smith, 1964; Biberstein, Nisbet and Thompson, 1967; Gilmour, 1978).

Pasteurella pneumonia has been recognised as one of the common sequelae to infection with C. phagocytophila (Foggie, 1951; Øveras, 1972; Foster and Cameron, 1970b). The objective of the present study, therefore, was to examine the effect of C. phagocytophila infection on the nasal carriage of P. haemolytica in sheep, and to investigate further the observation made by Brandreth (1978) that the number of sheep shedding P. haemolytica increased if sheep were infected with tick-borne fever.

MATERIALS AND METHODS

Animals

Two groups of sheep were used. One group consisted of nine-month-old sheep that were newly brought in from a neighbouring farm.

Three days after their arrival, each sheep was examined clinically and two swabs of nasal secretions were collected, one from each nostril. The second group consisted of acclimatised sheep which had been kept indoors for a period of one month. They were sampled 30 days after they were brought indoors. The sheep in both groups that yielded no P. haemolytica on the first occasion were again sampled a week later. Twenty-five newly bought and 13 acclimatised sheep from which P. haemolytica was not isolated on the two occasions were selected.

Inoculation

The newly bought and acclimatised sheep was subdivided into two further groups. Eleven of the newly bought sheep were inoculated intravenously with one ml of 10^{-1} dilution of TBF-infected (Old Sourhope strain of C. phagocytophila) blood while the remaining 14 were given one ml of sterile PBS by the intravenous route and served as controls. Six animals from the acclimatised group were inoculated intravenously with one ml of TBF-infected blood; the other seven sheep were each given one ml of sterile PBS by the intravenous route. The treatments are summarised below:-

Origin of sheep	TBF-infected	PBS injected
Newly bought	11	14
Acclimatised	6	7

Observations

The animals were observed for a period of four weeks after inoculation. Swabs of nasal secretions were taken from each nostril immediately before inoculation, and three and seven days after the

onset of the reaction. The controls were also sampled at the same time.

The sheep were bled for sera immediately prior to inoculation and thereafter at ten-day intervals.

Isolation of P. haemolytica

Cotton swabs attached to sticks, 25 cm long, were used to sample the nasal flora. Immediately before use the swabs were dipped into sterile nutrient broth. The external nares were wiped clean with gauze swabs moistened with sterile distilled water. To take samples the swab was passed through the nostril into the nasal passage until it contacted the naso-pharyngeal wall. The swab was moved gently back and forth and then withdrawn with a rotatory motion. Immediately after sampling, the swab was placed in sterile tube containing 0.5 ml of nutrient broth and broken free from its handle.

The isolation and identification of P. haemolytica were done according to the methods described by Osbaldiston (1973) and Cowan (1974). Each nasal swab was smeared over half the surface of a layered blood agar plate and then streaked out. The plates were incubated aerobically at 37°C for 18 hours. The plates were examined for colony formation; colonies resembling P. haemolytica were sub-inoculated onto fresh blood agar plates and incubated for 24 hours at 37°C. The primary isolation plates were incubated for a further 24 hours and any other P. haemolytica-like colonies were picked out and subcultured before the plates were discarded.

Smears of the cultures from the secondary isolation plates were prepared and stained by Gram's method. The Gram-stained smears were

examined under a light microscope; colonies of Gram-negative coccobacilli or short rods were subinoculated onto MacConkey agar plates and incubated at 37°C for 24 to 48 hours. Cultures which produced small colonies on MacConkey agar after 48 hours were inoculated into tryptose phosphate broth, tryptone water and onto blood agar plates and incubated at 37°C for 24 hours. The cultures were examined for their haemolytic and Gram-staining properties, motility, morphology, indole production, catalase and oxidase activities. Each isolate was also inoculated into sugar media to test its ability to ferment either trehalose or xylose.

Typing of P. haemolytica isolates

The serotypes of the P. haemolytica isolates were determined by the indirect haemagglutination (IHA) test (Biberstein and Thompson, 1966; Gilmour, personal communication).

Indirect haemagglutination test. Twenty-four hour blood agar cultures of P. haemolytica isolates were subcultured and grown overnight at 37°C in nutrient broth. The broth cultures were then heated at 56°C for 15 minutes to kill the bacteria.

Red blood cells from cattle were fixed in glutaraldehyde according to the method outlined by Shirai and his colleagues (1975). Fresh ox blood was collected into ten-ml evacuated tubes containing two ml of Alsever's solution, and centrifuged at 700 g for ten minutes. The plasma was discarded and the packed red blood cells were washed three times in PBS and resuspended to a concentration of 20 percent (V/V) in PBS. Equal volumes of a 0.2 percent glutaraldehyde in PBS and the 20 percent red blood cell suspension were

mixed, incubated at 37°C for 15 minutes and then centrifuged at 700 g for ten minutes. The glutaraldehyde-fixed red blood cells were washed five times and resuspended to a final concentration of ten percent (V/V) with PBS.

For the preparation of coated cells, ten ml of the ten percent glutaraldehyde-fixed red blood cell suspension was centrifuged at 700 g for ten minutes, the supernatant fluid was discarded and the pellet was washed with PBS before preparing a five percent suspension in PBS. Nine ml of the heated broth culture was added to one ml of the five percent suspension of fixed red blood cells and the mixture was incubated at 37°C for five minutes. After incubation, the sensitised red blood cells were washed three times in sterile PBS to remove excess antigen and then resuspended to the original volume with PBS.

The antisera against the twelve serotypes of P. haemolytica were inactivated at 56°C in a water-bath for 30 minutes and were used at a dilution of 1/30 except for serotype A_2 antiserum which was used at 1/4 dilution.

For the IHA test, a volume of 0.025 ml of the sensitised red blood cells was added to an equal volume of each of the diluted antiserum in U-type microtitre plates[1]. Non-sensitised ox red blood cells were added to each serum used in the test as control for non-specific agglutination. Red cells sensitised to each of the serotypes were used as antiserum controls. The plates were covered, gently agitated and allowed to stand at room temperature

[1]Cooke Microtitre System, Sterilin Products Ltd., Teddington, Middlesex

for two hours and then examined for agglutination. Positive reactions were indicated by the formation of a smooth mat of cells at the bottom of the well. Negative reactions produced compact 'buttons' of sedimented cells in the centre of the well.

Cultures of the isolates which reacted with more than one antisera were subinoculated onto blood agar plates and single colonies were used for antigen preparation in order to check whether such reaction was due to a mixture of serotypes in the original culture or due to cross reactions with more than one specific antisera.

Detection of serum antibodies to P. haemolytica

Titration of serum antibodies to P. haemolytica was done using the IHA test (Biberstein and Thompson, 1966; Gilmour, personal communication). Broth cultures of known P. haemolytica serotypes were subinoculated onto blood agar plates to check for purity. Single colonies of each serotype were used for antigen production. Glutaraldehyde-fixed ox red blood cells sensitised to a specific serotype were prepared as before. All serum samples were inactivated at 56°C for 30 minutes before use. Two-fold serial dilutions of the serum were made from 1/2 to 1/2048 in PBS in a volume of 0.025 ml in U-type microtitre plate. Equal volume of the sensitised red blood cells was added to each serum dilution. All serum samples were tested against the 12 serotypes. Non-sensitised red blood cells were incubated with the lowest dilution of each serum used as control for non-specific agglutination. The plates were held at room temperature for two hours and the result of the test was read

as before. The end-point dilutions were those showing 100 percent agglutination. The titres were expressed as the reciprocals of the end-point dilutions using a $\log_2$ series.

RESULTS

Clinical observations

The sheep that were inoculated with TBF-infected blood were febrile and parasitaemic three to four days after inoculation. One of the newly bought sheep developed respiratory distress and muco-purulent nasal discharges four days after the onset of fever. The other sheep made uneventful recoveries within two weeks after the onset of the reaction.

The control groups of newly bought and acclimatised sheep remained clinically normal throughout the observation period.

Isolation of P. haemolytica

Newly bought sheep. Pasteurella haemolytica was isolated from one (No. 191) out of 11 TBF-infected sheep six days after inoculation and from five sheep on day ten (Table 6.1). Serotype A_1 was isolated from one sheep on days six and ten; three sheep yielded serotype A_{11} and one sheep shed A_6 ten days after inoculation.

In contrast, only one out of the 14 newly bought, control sheep was found positive for P. haemolytica. The organism was isolated from the sheep six and ten days after saline inoculation and was found to be serotype A_{11} on both occasions (Table 6.2).

Acclimatised sheep. Pasteurella haemolytica was isolated from two out of the six TBF-infected sheep six days after inoculation and

from four sheep on day ten (Table 6.3). Serotype T_{10} was isolated from one sheep on days six and ten and from another sheep ten days after inoculation. One sheep yielded serotype A_7 on day six; two sheep shed serotype A_{11} ten days after inoculation.

In the control group of acclimatised sheep, two out of the seven sheep were positive for P. haemolytica ten days after saline inoculation. Serotype A_6 was isolated from one sheep and T_4 from the other sheep (Table 6.4).

An analysis of the bacterial isolations from the newly bought sheep revealed that the proportion of isolations from the TBF-infected animals was significantly higher than from the control sheep (Table 6.5). The proportion of isolations from the acclimatised TBF-infected sheep was also higher than from the control sheep but the difference only approached significance at the five percent level (Table 6.5).

The proportion of isolations from TBF-infected sheep that were newly bought was similar to the proportion of isolations from TBF-infected acclimatised sheep (Table 6.6). Moreover, the proportion of isolations from the control group of newly bought sheep was significantly lower than from the control group of acclimatised sheep (Table 6.6).

Detection of serum antibodies to P. haemolytica

Serum antibody titres to from two to nine serotypes of P. haemolytica were detected in serum samples collected immediately before inoculation (Appendix Tables 9-12).

Newly bought sheep. Five of the TBF-infected sheep that shed P. haemolytica showed a four-fold rise in antibody titres to the specific serotype isolated ten, 20 and 30 days after inoculation (Appendix Table 9). Two of these animals (No. 204 and 222) in addition developed rising titres to a specific serotype that was not isolated (Appendix Table 9).

In the control group, the sheep (No. 227) that shed P. haemolytica likewise showed rising antibody titres to the specific serotype isolated (Appendix Table 10). Two of the sheep (No. 225 and 100) from which the organism was not isolated also had increased antibody titres to a specific serotype ten, 20 and 30 days after saline inoculation (Appendix Table 10).

The antibody responses to specific serotypes in the newly bought sheep that shed P. haemolytica were dramatically higher than the antibody responses of the other newly bought sheep irrespective of whether they were infected with TBF or not (Fig. 6.1).

Acclimatised sheep. Similarly, four of the TBF-infected sheep that shed P. haemolytica developed a four-fold rise in antibody titres to the specific serotype isolated: one of these sheep (No. 190) also had rising titres to a specific serotype that was not isolated (Appendix Table 11).

In the control group, two of the sheep that shed P. haemolytica showed increased antibody titres to the specific serotype isolated during the period following saline inoculation (Appendix Table 12). One of the sheep (No. 195) from which P. haemolytica was not isolated also developed rising antibody titres to a specific serotype

(Appendix Table 12).

The antibody responses to specific serotypes in the acclimatised sheep that shed P. haemolytica were as before in marked contrast to the antibody responses of the other acclimatised sheep (Figure 6.2).

Comparisons. When the antibody titres of the sheep that shed P. haemolytica in the four experimental groups were subjected to a two-way Analysis of Variance significant interactions were detected at 0 and 20 days after infection (Appendix Table 13). The antibody titres of the shedding-infected sheep were consistently lower than the antibody titres of the shedding non-infected sheep (Table 6.7). None of the differences however was significant. Differences in antibody titres attributable to the origins of the sheep were also not significant (Table 6.8).

A comparison of the serum antibody assays of the newly bought sheep revealed that the proportion of sero-conversions from the TBF-infected sheep was significantly higher than from the control sheep (Table 6.9). The proportion of sheep with increased antibody titres in the acclimatised TBF-infected sheep and in the control group was similar (Table 6.9).

Likewise the proportion of sero-conversions from the two TBF-infected groups did not differ (Table 6.10). The proportion of sero-conversions from the control groups was also similar (Table 6.10).

DISCUSSION

Nasal carriage rate

Shreeve and Thompson (1970) investigated the nasal carriage of P. haemolytica in lambs. They found that the organism became established early in the respiratory flora of lambs. Strains of P. haemolytica, for example, were detected in clinically healthy lambs kept with their ewes within 12 days of birth, the first isolation being 48 hours after parturition had occurred. They observed that the isolation rate of individual strains of the organism increased as the lambs grew older; a concurrent widening of the serotype spectrum was also noted.

Biberstein and Thompson (1966) and Gilmour, Thompson and Fraser (1974) found P. haemolytica to be a part of the normal-bacterial flora of the nasopharynx and tonsils of adult sheep. Biberstein and his co-workers (1970) studied several flocks of normal sheep for nasal carriage of P. haemolytica. They observed a continuous and sometimes abrupt change in the relative frequencies of the various serotypes within a flock; they noted that the increase in carrier rates was often synchronous with the emergence of one type. They suggested that in the nasopharynx, it was possible that a constant competition existed between strains of P. haemolytica and the presence of a multiplicity of types and strains made it more likely that one of them would find a selective advantage in a changed set of circumstances. Epidemiological studies in sheep have shown changes in the carrier rate of P. haemolytica in normal flocks coinciding with the seasonal pattern of enzootic pneumonia (Biberstein et al., 1970) and have suggested multiplication of

P. haemolytica in the nasal passages and the possible spread of a particular strain through the flock during outbreaks of pneumonia (Biberstein and Thompson, 1966).

Brandreth (1978) observed that the number of sheep excreting P. haemolytica increased if the sheep were infected with tick-borne fever. She collected nasal secretions, sera and nasopharyngeal swabs from eight sheep before and ten days after inoculation with TBF-infected blood. P. haemolytica was isolated from one of the eight sheep prior to inoculation and from five sheep ten days after inoculation. Control sheep however were not examined concurrently.

In the present experiments, tick-borne fever affected the nasal carriage of P. haemolytica in groups of newly bought and acclimatised sheep. P. haemolytica was isolated from five out of 11 newly bought sheep ten days after inoculation with TBF-infected blood and from only one out of 14 control sheep, a proportion significantly lower than that of the TBF-infected sheep. In the acclimatised group, the proportion of isolations from the TBF-infected sheep was higher than from the control sheep although not statistically significant. Further analysis of the bacterial isolations revealed that the proportion of isolations from the newly bought TBF-infected sheep was similar to the proportions of isolations from the TBF-infected acclimatised sheep. On the other hand, the proportion of isolations from the control group of newly bought sheep was significantly lower than from the control group of acclimatised sheep. This finding was unexpected and is perhaps attributable to a poor experiment design in which the number of animals in each group was too small.

Thomson, Benson and Savan (1969) found that certain strains of P. haemolytica multiplied rapidly under altered environmental conditions. They not only noted a high rate of isolation but also a dramatic increase in the number of P. haemolytica in the nasal cavities of calves during the first two weeks after the calves had been transported; P. haemolytica was found absent or present only in low numbers in these calves prior to shipment. They postulated that the rapid increase in the number of P. haemolytica in the nasal passages eventually resulted in descent of the bacteria to the lungs in numbers sufficient to produce pneumonia. Grey and Thomson (1971) investigated the association between the numbers of P. haemolytica in the upper respiratory tract and their potential for deposition in the lung. Their observations suggested that P. haemolytica moved from the nose to the lung in the tracheal air in the form of droplet nuclei. They found that only calves positive for P. haemolytica on nasal swabs yielded the organism in the tracheal air. They concluded that once there was nasal colonisation by P. haemolytica the lung was exposed to extremely high numbers of the organism.

The bacterial isolations from the newly bought sheep indicated that proliferation of P. haemolytica in the nasal cavity coincided with the TBF parasitaemia. The isolations of the organism from the acclimatised sheep were independent of whether or not they were infected with tick-borne fever, suggesting that TBF alone was probably not responsible for the increased isolation rate of P. haemolytica from the newly bought sheep. It is not unlikely that tick-borne fever in combination with physical stress associated with

shipping incited multiplication of P. haemolytica in a greater number of newly bought sheep. Physical stress associated with movement and shearing has been considered as one of the factors involved in the initiation of septicaemic pasteurellosis in lambs (Biberstein and Kennedy, 1959).

The most common serotypes of P. haemolytica recovered by Biberstein and Thompson (1966) from normal sheep were A_2, A_7, A_{11} and A_{12}. Thompson and his colleagues (1977) found A_2 followed by A_1 and A_6 the serotypes most commonly isolated from pneumonic sheep. The T biotype of P. haemolytica was found mainly in septicaemia of older lambs whereas, the A biotype was predominantly found in pneumonia and septicaemia of very young lambs (Smith, 1961; Biberstein and Thompson, 1966). In the present study, serotypes A_1, A_6, A_7 and A_{11} but not A_2 and A_{12} were isolated; serotype A_{11} was the most frequent isolate. In addition, serotypes T_4 and T_{10} were also isolated.

Antibody response

Little has been reported concerning the levels of serum antibodies to P. haemolytica in sheep. Shreeve and Thompson (1970) found IHA antibodies to as many as five serotypes of P. haemolytica in the sera collected from lambs during the first two weeks of life and again after four weeks of age. In the ewes, antibodies to a broad spectrum of serotypes were detected at any one time. Antibodies corresponding to the serotypes carried by the ewes and lambs were found but often the strains of P. haemolytica isolated bore no relationship to the serum antibodies. Gilmour and his co-workers

(1980) demonstrated serum IHA antibody titres of three to eight to serotypes A_1 and A_6 in normal ewes.

Brandreth (1978) claimed that the increased isolation rate of P. haemolytica from sheep infected with TBF was associated with a rise in nasal rather than serum antibodies to the bacterium. Scrutiny of her data does not support this claim; the maximal changes in nasal antibody titres were only two-fold.

I detected antibodies to a wide range of P. haemolytica serotypes in the sera collected immediately before inoculation. However, during the period following inoculation, eight out of the 11 newly bought TBF-infected sheep developed a four-fold rise in antibody titres to specific serotypes, of these, five (62 percent) yielded P. haemolytica on nasal swabs. In the control group, only three out of 14 sheep developed increased antibody titres to specific serotypes; P. haemolytica was isolated from one (33 percent) of these sheep.

In the acclimatised TBF-infected group, four out of the six animals had increased antibody titres to specific serotypes; all were positive for P. haemolytica on nasal swabs. Three out of the seven acclimatised control sheep showed a rise in antibody titres to specific serotypes; P. haemolytica was isolated from two (66 percent) of these sheep.

A significant difference between the TBF-infected and non-infected newly bought sheep was found when the proportion of sero-conversions were analysed such that the proportion of sero-conversions in the newly bought TBF-infected sheep was higher than in the

newly bought non-infected group. This difference did not occur in the acclimatised sheep.

Four-fold rises in serum antibody titres to specific serotypes were detected in the newly bought and acclimatised sheep that shed P. haemolytica during the period following inoculation. In contrast, the antibody titres of the newly bought and acclimatised sheep from which the organism was not isolated were unchanged at ten, 20 and 30 days after inoculation.

The antibody titres of the TBF-infected sheep that shed P. haemolytica were consistently lower than the antibody titres of the non-infected shedders but the difference was not significant. There were also no significant differences in the antibody titres attributable to the origins of the sheep.

The mean serum antibody titres to specific serotypes in the non-infected sheep 20 and 30 days after saline inoculation, although lower, fell within the range of serum antibody titres reported by Gilmour and his colleagues (1980) to serotypes A_1 and A_6 in ewes.

Earlier, we showed that antibody responses to a clostridial vaccine were suppressed in sheep clinically affected with TBF (Chapter Four). In the present study, the antibody titres to specific serotypes of P. haemolytica in the TBF-infected sheep that shed the bacteria were found to be lower than the antibody titres of the shedding non-infected sheep in the early stages of the immune response which tempts the suggestion that this difference was associated with the TBF-induced immunosuppression.

Postulation

The finding that multiplication of P. haemolytica in the nasal cavities occurred during TBF supports the postulated link between TBF and outbreaks of Pasteurella pneumonia in sheep (Foggie, 1951; Øveras, 1972).

Biberstein and Thompson (1965) stated that the pathogenic process of P. haemolytica consisted of a build-up in the host of a bacterial population that will be toxic and that the virulence of strains of P. haemolytica was due to their ability to grow rapidly from a small inoculum to a toxic concentration. In a series of experiments Green and Kass (1964a; 1964b) showed that the bacterial clearance from the lung was dependent on alveolar macrophages and was influenced by various diverse factors. Jakab and Green (1972) found that Sendai virus infection inhibited the bactericidal activity against Staphylococcus aureus in the lung and permitted the multiplication of the bacteria. Lopez and his colleagues (1976) reported that inhibition of the bactericidal activity of the alveolar macrophages for P. haemolytica by parainfluenza-3 virus may be a key factor in the pathogenesis of pneumonic pasteurellosis in calves.

Snodgrass (1974) demonstrated that C. phagocytophila was present in the lungs of sheep 24 hours after the intravenous inoculation of infected blood; during the reaction high titres of the rickettsias were consistently recorded from the lungs. The organism was also detected in alveolar macrophage. It is possible that during its multiplication in the lung, C. phagocytophila induced damage to the tissues or to the alveolar macrophages sufficient to render the lower respiratory tract more susceptible to bacterial invasion. It

was shown in a previous experiment (Chapter Five) that the phagocytic activity of neutrophil leucocytes was reduced during the TBF parasitaemia; the reduction in phagocytosis was attributed to the limited phagocytic activity of parasitised neutrophils.

It is possible, therefore, that infection with C. phagocytophila increases the susceptibility of sheep to secondary Pasteurella pneumonia through primary rickettsial damage of alveolar macrophages and through its immunosuppressive effect.

Table 6.1 Isolation of *Pasteurella haemolytica* from newly bought TBF-infected sheep

Sheep No.	Serotype isolated	Days after inoculation	
		6	10
184		-	-
186		-	-
188		-	-
189		-	-
191	A_1	+	+
203	A_{11}	-	+
204	A_{11}	-	+
222	A_6	-	+
223	A_{11}	-	+
101		-	-
103		-	-

Table 6.2 Isolation of *Pasteurella haemolytica* from newly bought control sheep

Sheep No.	Serotype isolated	Days after inoculation	
		6	10
183		-	-
185		-	-
224		-	-
225		-	-
226		-	-
227	A_{11}	+	+
100		-	-
102		-	-
193		-	-
194		-	-
182		-	-
201		-	-
202		-	-
208		-	-

Table 6.3 Isolation of *Pasteurella haemolytica* from acclimatised TBF-infected sheep

Sheep No.	Serotype isolated	Days after inoculation	
		6	10
205		-	-
206	T_{10}	-	+
207	T_{10}	+	+
	A_{11}	-	+
190	A_7	+	-
192	A_{11}	-	+
187		-	-

Table 6.4 Isolation of *Pasteurella haemolytica* from acclimatised control sheep

Sheep No.	Serotype isolated	Days after inoculation	
		6	10
180	A_6	-	+
181	T_4	-	+
195		-	-
220		-	-
221		-	-
228		-	-
229		-	-

Table 6.5 Isolation of *Pasteurella haemolytica* from nasal swabs taken from newly bought sheep and acclimatised sheep

Experimental Group		No. of sheep	Positive	Chi-square
Newly bought sheep	TBF-infected	11	5	7.280**
	control	14	1	
Acclimatised sheep	TBF-infected	6	4	3.730
	control	7	2	

** $p < 0.010$

Table 6.6 Isolation of *Pasteurella haemolytica* from nasal swabs taken from TBF-infected and control sheep

Experimental group		No. of sheep	Positive	Chi-square
TBF-infected	Newly bought sheep	11	5	1.811
	Acclimatised sheep	6	4	
Control	Newly bought sheep	14	1	3.937*
	Acclimatised sheep	7	2	

* $P < 0.050$

Table 6.7 Mean serum antibody titres ($\log_2$ reciprocal) and standard deviations of nine TBF-infected and three non-infected sheep that shed P. haemolytica

Days after inoculation	Infected Sheep	Non-infected sheep
0	0.7 ± 0.8	1.0 ± 1.0
10	2.7 ± 0.9	3.3 ± 1.5
20	4.1 ± 0.6	4.7 ± 1.2
30	4.3 ± 0.9	4.0 ± 0.0

Table 6.8 Mean serum antibody titres ($\log_2$ reciprocal) and standard deviations of six newly bought sheep and six acclimatised sheep that shed *P. haemolytica*

Days after inoculation	Newly bought sheep	Acclimatised sheep
0	0.5 ± 0.8	1.0 ± 0.8
10	3.2 ± 1.2	2.6 ± 1.0
20	4.5 ± 0.8	4.0 ± 0.6
30	4.5 ± 0.5	4.0 ± 1.0

Table 6.9 Serum antibody response to *Pasteurella haemolytica* of newly bought sheep and acclimatised sheep

Experimental group		No. of sheep	No. of sheep with a four-fold increase in antibody titre	Chi-square
Newly bought sheep	TBF-infected	11	8	8.825**
	Control	14	3	
Acclimatised sheep	TBF-infected	6	4	2.006
	Control	7	3	

** $P < 0.010$

Table 6.10 Serum antibody response to *Pasteurella haemolytica* of TBF-infected and control sheep

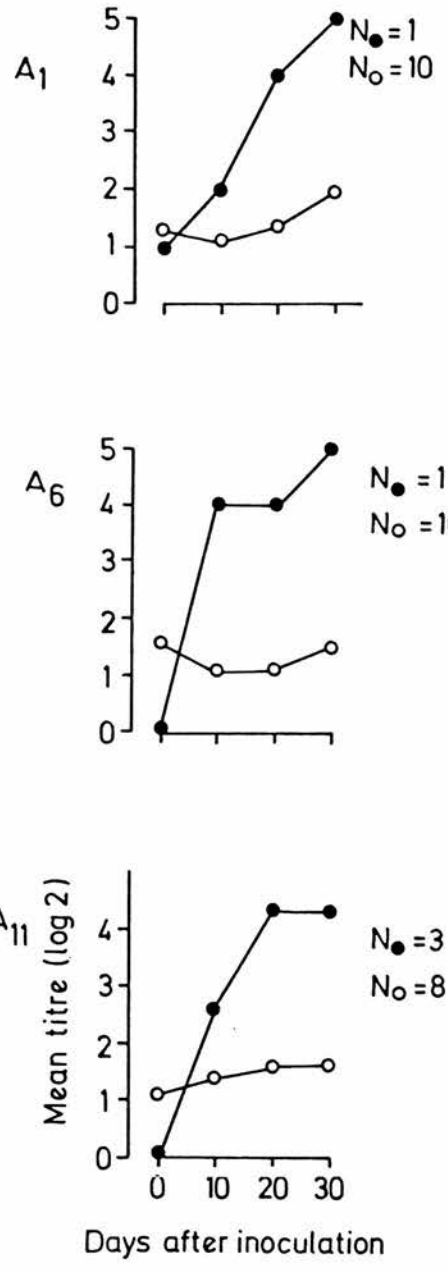
Experimental group		No. of sheep	No. of sheep with a four-fold increase in antibody titre	Chi-square
TBF-infected	Newly bought sheep	11	8	0.087
	Acclimatised sheep	6	4	
Control	Newly bought sheep	14	3	2.362
	Acclimatised sheep	7	3	

Fig. 6.1 Serum antibody responses to specific *P. haemolytica* serotypes in newly bought sheep

● Shedder

○ Non-shedder

Newly bought TBF-infected sheep



Newly bought control sheep

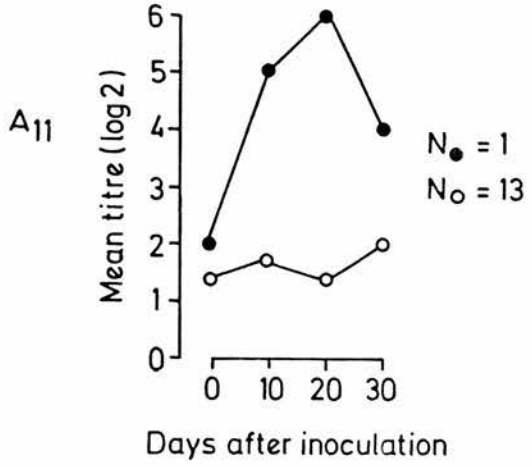
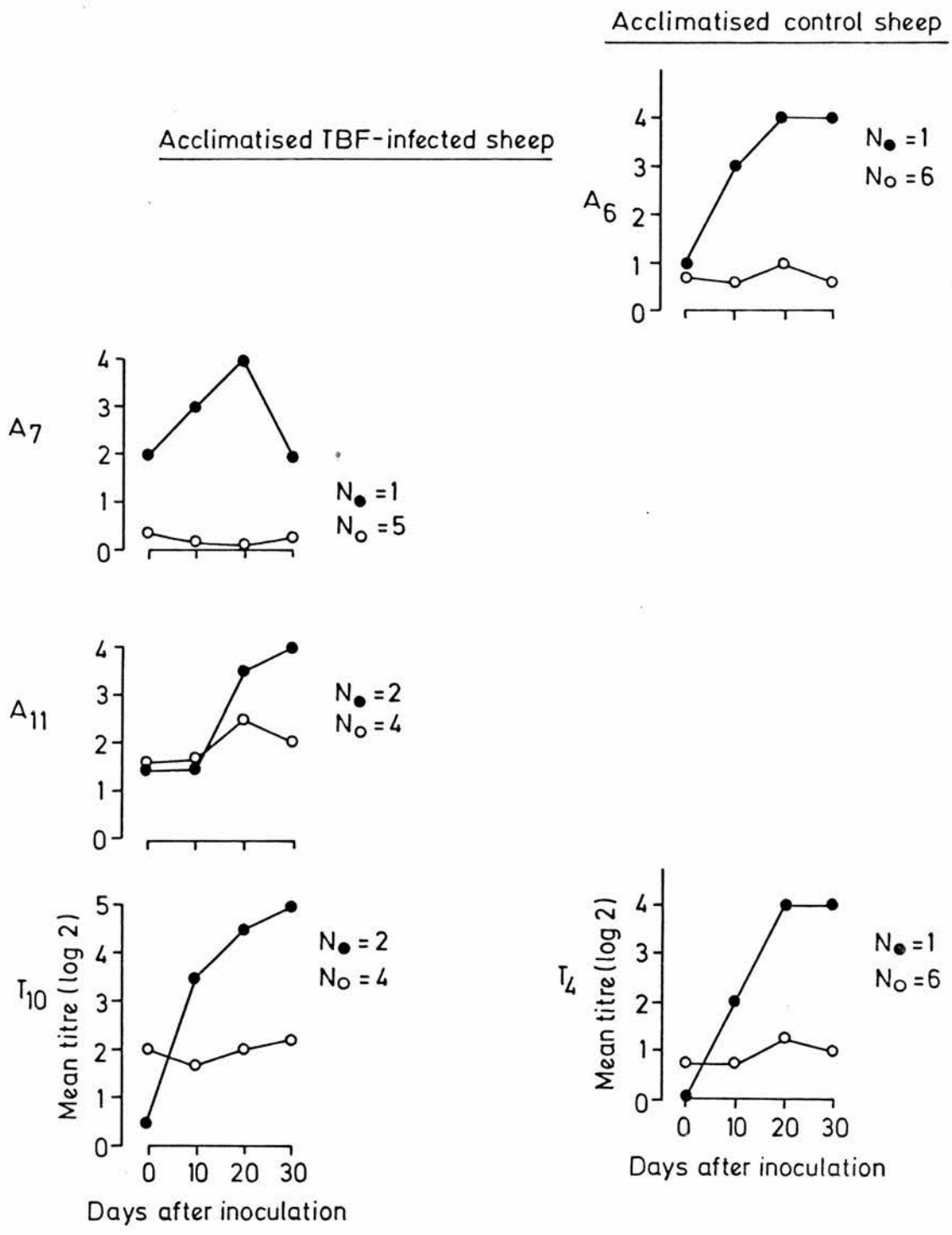


Fig. 6.2 Serum antibody responses to specific *P. haemolytica* serotypes in acclimatised sheep

● Shedder

○ Non-shedder


Acclimatised control sheep
Acclimatised TBF-infected sheep
A6
A7
A11
T10
T4
Mean titre (log 2)
N● = 1
No = 6
N● = 1
No = 5
N● = 2
No = 4
N● = 2
No = 4
N● = 1
No = 6
0 10 20 30
Days after inoculation

CHAPTER SEVEN

TICK-BORNE FEVER AND CONCURRENT PARAINFLUENZA-3 VIRUS INFECTED IN SHEEP

The annual reports of the East of Scotland College of Agriculture have for years considered the most common cause of death in sheep in areas in the east of Scotland to be pneumonia. In the known tick-infested areas, many of the incidents of pneumonia in young lambs were found to be related to concurrent infections with TBF (Anon., 1971; 1974; 1978).

Serological surveys have shown that infection with parainfluenza-3 (PI-3) virus is widespread in the sheep population (Hore, 1969; Karrar, 1977). The virus moreover has been associated with ovine pneumonia but experimental infections with PI-3 virus alone have consistently produced only mild respiratory disease; it has been shown however that infection with PI-3 virus in combination with other agents induces severe clinical reactions and pneumonia in sheep (Hore and Stevenson, 1969; Biberstein, Shreeve, Angus and Thompson, 1971; Davies, Dungworth, Humphreys and Johnson, 1977; Sharp et al., 1978).

The present study was designed to examine the effect of TBF on concurrent PI-3 virus infection and to obtain further evidence on the association of TBF with outbreaks of pneumonia in sheep postulated by Foggie (1951) and Øveras (1972).

MATERIALS AND METHODS

Animals

Fifty lambs of several breeds and their crosses reared on tick-free farms in Scotland were used. Most were females or castrated males, six to nine months old. None showed clinical or serological evidence of previous infection with PI-3 virus. The lambs were housed for the duration of the experiment in isolation rooms in which the ambient temperatures were maintained at 22°C.

Rickettsia

The Old Sourhope strain of *C. phagocytophila* was used. Immediately prior to inoculation, a cryopreserved aliquot of a stabilate of TBF-infected blood was rapidly thawed in running water and diluted 10^{-1} and 10^{-3} with sterile PBS.

Virus

The PI-3 virus was an ovine strain isolated by Dr. D.R. Snodgrass of the Moredun Institute. The virus was subpassaged four times and titrated in secondary lamb testes cell (LTC) culture maintained in Eagle's minimum essential medium supplemented with three percent heat-inactivated new-born calf serum, ten percent phosphate broth and antibiotics[1]. The infective cell culture fluid which had a titre of $10^{6}TCID_{50}$ per ml of PI-3 virus was dispensed into two-ml aliquots and stored at -114°C in the vapour-phase of a liquid nitrogen refrigerator.

[1]Penicillin 100,000 units/ml and streptomycin 50,000 µg/ml

Inoculation

The lambs were ear-tagged and then allocated randomly into five groups of ten by drawing their numbers from a hat. Group A was inoculated intranasally with two ml of the cell culture fluid containing approximately $10^6 TCID_{50}$ per ml of PI-3 virus. Lambs in group B were each inoculated simultaneously with the same dose of PI-3 virus intranasally and with one ml of the 10^{-1} dilution of TBF-infected blood by the intravenous route. Group C was inoculated simultaneously with two ml of the PI-3 virus suspension by the intranasal route and intravenously with one ml of the 10^{-3} dilution of TBF-infected blood. Lambs in Group D were each given one ml of the 10^{-1} dilution of TBF-infected blood by the intravenous route and then later inoculated intranasally with two ml of the PI-3 virus suspension at the onset of TBF parasitaemia. Group E was inoculated intravenously with one ml of the 10^{-1} dilution of TBF-infected blood alone. The treatments are summarised below:-

Experimental group	10^{-1} TBF on day 0	10^{-3} TBF on day 0	PI-3 on day 0	PI-3 at onset of TBF parasitaemia
A			+	
B	+		+	
C		+	+	
D	+			+
E	+			

Observations

The lambs were observed thereafter for clinical manifestations of PI-3 virus and TBF infections. Rectal temperatures were read

daily before blood samples were taken and each nostril was swabbed separately. The animals were bled for sera before and after virus inoculation at weekly intervals.

Animals which died during the experimental period were taken to the post-mortem room and were examined for gross lesions. Lung and bronchial lymph nodes were sampled for bacteria and viruses. Representative portions of the lung lesions were taken for histological examination by Mr. J.S.D. Ritchie of the Department of Veterinary Pathology.

Bacterial culture

Lung and bronchial lymph node tissues were inoculated onto blood agar plates and incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified on the basis of their colonial morphology, Gram-staining and biochemical properties (Osbaldiston, 1973; Cowan, 1974).

Virus isolation

The isolation of PI-3 virus from the nasal swabs and lung tissue was done according to the methods described by Grist, Ross and Bell (1974). Immediately after collection, the nasal swabs were placed in sterile tubes containing two ml HBSS containing antibiotics[1]. The nasal swab suspension was centrifuged at 700 g for ten minutes at 4°C. The supernatant fluid was collected and then passed through a 0.22 µm membrane filter attached to a syringe[2].

[1]Penicillin 400 units/ml, streptomycin 400 µg/ml and gentamicin 20 µg/ml

[2]Millex filter, Millipore Corp., Bedford, Mass.

A volume of 0.2 ml of the filtered material was inoculated into tubes of confluent secondary LTC culture on flying coverslip. Four tubes were used per sample.

Extracts of the lung tissues were prepared by grinding pieces in a mortar and then adding sterile Hanks' solution containing antibiotics to make a ten percent suspension. After centrifugation at 700 g for 15 minutes at 4°C, the supernatant fluid was collected and 0.1 ml was immediately inoculated into tubes of LTC monolayer.

All inoculated tubes were incubated at 37°C for one hour to allow for virus adsorption. The inoculum was removed and the cell culture was rinsed with sterile PBS before one ml of maintenance medium was added to each tube. Duplicate tubes of uninoculated LTC culture were set aside as cell controls. Tubes were incubated at 37°C in stationary racks and were observed for the development of cytopathic effects (CPE).

On the sixth day of incubation, CPE was assessed by examining each tube of inoculated LTC culture under an inverted microscope. Tubes showing little CPE were tested for haemadsorption.

For the haemadsorption test, a volume of 0.2 ml of a 0.5 percent guinea pig red blood cell suspension was added to each tube after the cell culture fluid had been removed. The tubes were placed on a horizontal position and incubated at 4°C in a refrigerator for 30 minutes. Unadsorbed red blood cells were removed by washing the cell monolayers twice with cold PBS. The cell cultures were then examined for the presence of haemadsorption using an inverted microscope.

The nature of the CPE was further examined on Giemsa-stained cell cultures on flying coverslips which were removed and fixed with methanol for 15 minutes. They were then stained with ten percent Giemsa stain for 30 minutes, rinsed in tap water and differentiated in buffer for two minutes. The stained coverslips were placed in xylene for 20 minutes, mounted in DPX[1] on microscope slides and examined under a light microscope.

From cultures which showed CPE and haemadsorption 0.2 ml of cell culture fluid and cells were passed into further cultures, and incubated for three days. The cell cultures were harvested by one cycle of freezing and thawing and pools of the infected culture fluids were distributed into sterile ampoules and stored at -114°C until required for tests to identify the virus isolate.

Cell cultures which showed little CPE were washed with sterile PBS before fresh maintenance medium was added and re-incubated for two to three days. A sample was considered to be negative for virus if CPE failed to develop after two serial subpassages in cell cultures.

Serum antibody assay

The serum antibody titres to PI-3 virus were measured using the haemagglutination-inhibition (HI) and virus neutralisation (VN) test (Grist, Ross and Bell, 1974).

Haemagglutination-inhibition test. Prior to testing, the sera were treated for the removal of non-specific, red cell agglutinins

[1]George Gurr Ltd., London

and non-specific inhibitors of haemagglutination. The sera were inactivated at 56°C in a water-bath for 30 minutes. After they had cooled, an equal volume of a 25 percent suspension of kaolin in PBS was added to each sample to remove non-specific inhibitors of haemagglutination. The serum-kaolin mixtures were held at room temperature for 20 minutes and then centrifuged at 700 g for 15 minutes. One part of packed guinea pig red blood cells (RBC) was added to four parts of the kaolin-treated serum to remove non-specific red cell agglutinins. The mixtures were incubated at 4°C overnight and then centrifuged at 700 g for 15 minutes. The treated sera were diluted with PBS and used at an initial dilution of 1/4.

The haemagglutination-inhibition test was done in V-type micro-titre plates[1] using four haemagglutinin (HA) units of the PI-3 virus antigen[2] and a 0.5 percent suspension of guinea pig RBC. The titre of the antigen was determined on the day of the test by haemagglutination titration. Two-fold serial dilutions of the serum samples were made in a volume of 0.025 ml in PBS using microdiluters. A volume of 0.025 ml of the antigen suspension containing four HA units of PI-3 virus was added to each serum dilution. A negative serum control, virus control dilution at four, two, one and half HA units of antigen, and serum control containing the lowest dilution of the serum were set up at the same time. The plates were covered and shaken gently during incubation at 37°C. After one hour of

[1]Cooke Microtitre System, Sterilin Products Ltd., Teddington, Middlesex

[2]Flow Laboratories, Irvine, Ayrshire

incubation, a volume of 0.05 ml of a 0.5 percent suspension of guinea pig RBC was added to each well; a red blood cell control well was included. The plates were again shaken and then held at room temperature for one hour. The test was read when the cells in the RBC control well had settled into a solid 'button'. The end-point of the reaction was taken as the highest dilution of the serum which completely inhibited haemagglutination by four HA units of virus. The titres were expressed as the reciprocals of the end-point dilutions using a $\log_2$ series.

Virus neutralisation test. Vero cell monolayers were grown out in the wells of sterile disposable flat-bottomed microtitre plates[1]. Approximately 16,000 Vero cells in 0.2 ml of Eagle's minimum essential medium supplemented with five percent heat-inactivated foetal-calf serum and antibiotics were added into each well. The plates were covered, sealed and incubated at 37°C. Confluent monolayers were obtained by 24 hours.

Serial two-fold dilutions of the heat-inactivated pooled serum were made in PBS and combined with an equal volume of PI-3 virus suspension containing approximately 100 $TCID_{50}$ per 0.1 ml. The virus-serum mixtures were incubated for one hour at room temperature. The growth medium was removed before 0.1 ml of the virus-serum mixtures were added to each well. Eight wells of Vero cell monolayer were used per serum dilution. Ten-fold dilutions of the virus control (100, 10, 1.0, 0.1 and 0.01 $TCID_{50}$) were prepared at the same time and 0.1 ml of each dilution was inoculated into four wells

[1]Nunclon, Nunc InterMed, Denmark

of cell culture. Serum controls containing the lowest dilution of the serum samples were included to check for toxicity of the sera. Uninoculated wells of cell culture were set aside as cell controls. A volume of 0.2 ml of fresh maintenance medium was then added to each well. The plates were covered, sealed and incubated at 37°C. After four days of incubation, the cell cultures were examined for the presence of CPE using an inverted microscope. The cell culture fluid was then tipped off and the cells were fixed with ten percent buffered formalin for five minutes and stained with 0.1 percent solution of crystal violet for ten minutes (Grimes, King and Kleven, 1976). The cell cultures were then washed gently with tap water and the test was finally read. A cytopathic effect was indicated by cell degeneration and clear areas in the stained cell culture whereas virus neutralisation was indicated by the presence of evenly stained confluent cell monolayer. The serum antibody titre was the highest dilution of the serum in the initial serum-virus mixture which protected 50 percent of the cell cultures against 100 $TCID_{50}$ of virus. The serum and virus titrations were calculated by the Spearman-Karber method (Finney, 1952).

Identification of virus isolates

The virus isolates were identified by HI and VN tests using specific PI-3 virus antiserum[1] (Grist, Ross and Bell, 1974).

Tube neutralisation test. The cell culture fluid containing the virus isolate was diluted 1/10 with sterile PBS. Equal volumes

[1]Flow Laboratories, Irvine, Ayrshire

of the virus suspension and the 1/40 dilution of the reference antiserum were combined and held at room temperature for one hour. A volume of 0.2 ml of the serum-virus mixture was inoculated into LTC cultures. Two cell cultures were inoculated with the virus suspension and two uninoculated cell cultures were set aside as virus and cell controls respectively. The cultures inoculated with the serum-virus mixture were examined 24 hours after the virus controls showed complete degeneration of the cell cultures. The haemadsorption test was used as indicator of non-neutralised virus.

Haemagglutination-inhibition test. The haemagglutinating activity of the virus isolates was first determined. Two-fold serial dilutions of the cell culture fluid containing the virus isolate were made in PBS in a volume of 0.025 ml in the wells of V-type microtitre plate. Parallel dilutions of known PI-3 virus suspension were prepared at the same time. A volume of 0.05 ml of a 0.5 percent suspension of guinea pig RBC was added to each well; a red blood cell control well was also included. The plate was shaken and then held at room temperature for one hour. The test was read when the cells in the RBC control well had sedimented into a solid 'button' and the virus control wells showed complete haemagglutination. The highest dilution of the virus isolate that produced complete haemagglutination was taken to equal one haemagglutinin (HA) unit.

The identity of the virus isolates which showed haemagglutinating activity were then confirmed by haemagglutination-inhibition test using specific PI-3 virus antiserum of known HI titre and four HA units of the virus isolate.

RESULTS

Clinical course

The lambs that were infected with TBF alone had few clinical signs other than fever. The lambs inoculated with PI-3 virus alone had low-grade fevers together with mild, transient respiratory signs. In contrast, the lambs infected simultaneously with both agents had higher fevers and showed respiratory distress. The severity of the clinical reactions was even greater in lambs infected with PI-3 virus at the onset of the TBF parasitaemia; two of these lambs became progressively ill and died eight (sheep No. 96) and 15 days (sheep No. 95) after the onset of the febrile reactions.

Mr. J.S.D. Ritchie necropsied the two lambs and found that they had severe antero-ventral pneumonia of the mixed Pasteurella-PI-3 virus type with grey solid cellular lung lesions and mild pleurisy. The spleen and carcase nodes did not show well developed TBF-lesions. On histological examination, the lungs showed a mixed pattern of typical exudative Pasteurella pneumonia lesions with multinucleate syncytia present. There was little cuffing response but alveolar epithelialisation was prominant in areas. No well developed syncytial intracytoplasmic or intranuclear inclusions were seen. The lung lesions were compatible with the macroscopic mixed Pasteurella and PI-3 virus pneumonia pattern.

Bacterial culture

I isolated P. haemolytica from the lungs of the two lambs that died.

Fever

Group responses. Group A. Seven out of the ten lambs that were inoculated with PI-3 virus alone were pyrexic three days after inoculation; all had temperatures of over 40.5°C on day four (Figs. 7.1, 7.2, 7.3). The mean incubation period was 2.3 ± 0.4 days in the range two to three days (Table 7.1). A mean maximal temperature of 41.0 ± 0.3°C was recorded 1.2 ± 1.0 days after the onset of the febrile reaction which lasted for 4.0 ± 0.9 days in the range three to five days (Table 7.1). The magnitude of the febrile reactions expressed in terms of the area bounded by the plot of the daily rectal temperatures on a five mm grid was 1,150 ± 234 mm^2 (Table 7.1).

Group B. The lambs that were inoculated simultaneously with the 10^{-1} dilution of TBF-infected blood and PI-3 virus had a mean incubation period of 2.4 ± 0.8 days in the range one to four days (Table 7.1). A mean peak temperature of 41.9 ± 0.2°C was observed 1.4 ± 1.4 days after the onset of the fever which persisted for 7.5 ± 1.2 days in the range six to nine days (Table 7.2). The mean magnitude of the febrile responses was 2,758 ± 896 mm^2 (Table 7.2).

Group C. The group that was inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus had a mean incubation period of 3.0 ± 1.1 days in the range two to five days (Fig. 7.2). A mean maximal temperature of 41.9 ± 0.3°C was noted 1.8 ± 1.2 days after the onset of the febrile reaction which lasted for 8.5 ± 1.7 days in the range five to 11 days (Table 7.3). The mean magnitude of the febrile reactions was 3,295 ± 868 mm^2 (Table 7.3).

Group D. The lambs that were inoculated with the 10^{-1} dilution of the TBF-infected blood and later exposed to PI-3 virus at the onset of the TBF parasitaemia had a mean incubation period of 2.5 ± 0.5 days in the range two to three days (Fig. 7.3). A mean peak temperature of 42.0 ± 0.2°C was recorded 1.0 ± 0.8 day after the onset of fever which lasted for 9.3 ± 1.8 days in the range six to 12 days (Table 7.4). The mean magnitude of the febrile reactions was 3,698 ± 687 mm^2 (Table 7.4).

Group E. The lambs that were inoculated with the 10^{-1} dilution of TBF-infected blood alone were febrile one to three days after inoculation, the mean incubation period being 2.0 ± 0.6 days (Figs. 7.1, 7.2, 7.3). A mean maximal temperature of 41.7 ± 0.2°C was observed 1.7 ± 1.4 days after the onset of fever which persisted for 6.6 ± 2.5 days in the range four to 12 days (Table 7.5). The mean magnitude of the febrile responses was 2,108 ± 614 mm^2 (Table 7.5).

Comparisons. The mean incubation period in the group inoculated with PI-3 virus alone was similar to that of the group infected with TBF alone and to those of the groups with dual infections (Table 7.6).

The mean maximal temperature in the lambs inoculated with PI-3 virus was significantly lower than the mean peak temperatures in the group of lambs infected with TBF alone and the groups inoculated with both agents (Table 7.6). The day on which maximal temperatures occurred was similar in all groups (Table 7.6).

The mean duration of the febrile reactions was significantly shorter in the lambs infected with PI-3 virus alone than in the groups with dual infections and the group of lambs infected with TBF alone (Table 7.6). The mean durations of the febrile reactions in the other groups fell into two overlapping subsets, the longest duration being 8.4 ± 1.6 days for the subset containing all the dual infections (Table 7.6).

Further analysis confirmed that the febrile responses in the lambs inoculated with PI-3 virus alone were significantly lower than the responses in the other groups (Table 7.6). The mean magnitudes of the fevers in the lambs in the other experimental groups fell into three overlapping subsets, the mean magnitudes being respectively 2,400 ± 600 mm^2 for the subset containing the group infected with TBF alone and the group inoculated simultaneously with the 10^{-1} dilution of TBF-infected blood and PI-3 virus, 3,000 ± 900 mm^2 for the subset containing the groups that were inoculated simultaneously with both agents and 3,500 ± 800 mm^2 for the subset containing the group inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus and the group that was exposed to the virus at the onset of the TBF parasitaemia (Table 7.6).

Haematology

Group Responses. Group A. In the group inoculated with PI-3 virus alone, the changes in the mean total leucocyte count were slight and statistically non-significant (Table 7.7).

The mean lymphocyte count increased after inoculation, the magnitude of the lymphocytosis being 141.6 ± 136.4 mm^2 (Table 7.8,

Figs. 7.4, 7.8, 7.12, Appendix Table 14); the rises however were not significant (Table 7.9). A tendency toward a lymphocytopaenia (Figs. 7.4, 7.8, 7.12, Appendix Table 14) was noted 6.5 ± 3.1 days after inoculation when the mean lymphocyte count fell from a pre-inoculation level of $6.1 \times 10^9 \pm 0.7 \times 10^9$ per l to a mean nadir of $4.8 \times 10^9 \pm 0.9 \times 10^9$ per l (Table 7.9 and 7.10). The mean magnitude of the lymphocytopaenia was 372.1 ± 341.5 mm^2 (Table 7.10).

There was also a non-significant increase in the number of neutrophils following inoculation, the mean magnitude of the neutrophilia being 241.5 ± 323.7 mm^2 (Table 7.11, Figs. 7.5, 7.9, 7.13, Appendix Table 15, Table 7.12). During the reaction, the decrease in the number of neutrophils was minimal; the mean magnitude of the neutropaenia was 410 ± 339.6 mm^2 (Figs. 7.5, 7.9, 7.13, Appendix Table 15, Table 7.12).

The number of eosinophils also decreased during the reaction (Figs. 7.6, 7.10, 7.14, Appendix Table 16); the eosinopaenia however was statistically significant only on day six (Table 7.13). The magnitude of the eosinopaenia was 1,411.4 ± 815.5 mm^2 (Table 7.14).

The monocyte counts showed minimal changes early in the reaction. Later the number of monocytes increased gradually and reached significant levels 12 to 14 days after virus inoculation (Table 7.15, Figs. 7.7, 7.11, 7.15, Appendix Table 17). The mean magnitude of the monocytosis was 347.3 ± 229 mm^2 (Table 7.14).

Group B. The mean daily total leucocyte counts in the group inoculated simultaneously with the 10^{-1} dilution of TBF-infected

blood and PI-3 virus increased after inoculation (Table 7.16). The leucocytosis however was not significant and of short duration being immediately succeeded by a statistically significant leucopaenia (Table 7.16). The mean total leucocyte count fell from a pre-inoculation level of $10.3 \times 10^9 \pm 1.4 \times 10^9$ per l to a mean nadir of $4.7 \times 10^9 \pm 0.8 \times 10^9$ per l, 10.1 ± 3.3 days after inoculation (Table 7.16 and 7.17).

There was a significant increase in the mean lymphocyte count on the day after inoculation, the magnitude of the lymphocytosis being 43.5 ± 64.8 mm^2 (Table 7.18). The lymphocytosis was brief and was followed by an abrupt fall in the number of lymphocytes (Fig. 7.4, Appendix Table 18). The mean lymphocyte count dropped from a pre-inoculation level of $7.0 \times 10^9 \pm 0.9 \times 10^9$ per l to a mean nadir of $2.3 \times 10^9 \pm 0.6 \times 10^9$ per l, 6.2 ± 1.3 days after inoculation (Tables 7.19 and 7.20). The mean magnitude of the lymphocytopaenia was 3,368.9 ± 618.9 mm^2 (Table 7.20).

The mean neutrophil count increased significantly from a pre-inoculation level of $2.6 \times 10^9 \pm 0.5 \times 10^9$ per l to a maximal mean count of $4.7 \times 10^9 \pm 1.4 \times 10^9$ per l, 3.5 ± 1.0 days after inoculation (Tables 7.21 and 7.22). The mean magnitude of the neutrophilia was 641.8 ± 402.3 mm^2 (Table 7.22). The neutrophilia lasted five days and was succeeded by a period of profound neutropaenia (Table 7.21, Fig. 7.5, Appendix Table 19). The number of neutrophils dropped suddenly to a mean nadir of $0.7 \times 10^9 \pm 0.2\ 10^9$ per l, 8.4 ± 2.4 days after the onset of visible parasitaemia (Table 7.23). The magnitude of the neutropaenia was 2,913.7 ±

764.1 mm^2 (Table 7.23).

There was a significant and sudden drop in the number of eosinophils on the day after the onset of visible parasitaemia (Table 7.24, Fig. 7.6, Appendix Table 20). The eosinopaenia was evident for nine days, the mean magnitude being 4,170 ± 1,011 mm^2 (Tables 7.24 and 7.25).

The number of monocytes rose significantly six days after inoculation (Table 7.26, Fig. 7.7, Appendix Table 21). The monocytosis lasted six days occurring on the fifth to the tenth day of the parasitaemia. The mean magnitude of the monocytosis was 2,043.3 ± 1,070.7 mm^2 (Table 7.25).

Group C. In the group that was inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus, the mean total leucocyte count likewise rose after inoculation but the increase in the total number of leucocytes was statistically non-significant (Table 7.27). Subsequently, the mean total leucocyte count fell from a pre-inoculation level of $10.3 \times 10^9 \pm 1.6 \times 10^9$ per l to a mean nadir of $4.7 \times 10^9 \pm 0.9 \times 10^9$ per l, 8.3 ± 2.1 days after inoculation (Tables 7.27 and 7.28).

The differential white cell counts showed a similar trend. There was a significant increase in the number of lymphocytes on the day after inoculation, the mean magnitude of the lymphocytosis being 32.3 ± 51.3 mm^2 (Tables 7.29 and 7.30). The lymphocytosis was immediately followed by a marked decrease in the number of lymphocytes (Fig. 7.8, Appendix Table 22). The mean lymphocyte count fell from a pre-inoculation level of $7.2 \times 10^9 \pm 1.2 \times 10^9$

per l to a mean nadir of $2.3 \times 10^9 \pm 0.6 \times 10^9$ per l, 7.5 ± 2.4 days after inoculation (Tables 7.29 and 7.31). The mean magnitude of the lymphocytopaenia was 3,194 ± 899.4 mm^2 (Table 7.31).

The number of neutrophils rose significantly on the day after inoculation from a pre-inoculation level of $2.4 \times 10^9 \pm 0.6 \times 10^9$ per l to a maximal mean count of $3.7 \times 10^9 \pm 1.4 \times 10^9$ per l, 4.2 ± 2.4 days after inoculation (Tables 7.32 and 7.33). The mean magnitude of the neutrophilia was 506 ± 390.7 mm^2 (Table 7.33). The mean neutrophil count remained elevated for six days and then fell dramatically (Fig. 7.9, Appendix Table 23) reaching a mean nadir of $0.8 \times 10^9 \pm 0.5 \times 10^9$ per l, 8.8 ± 3.3 days after the onset of visible parasitaemia (Table 7.34). The mean magnitude of the neutropaenia was 2,135.4 ± 947.6 mm^2 (Table 7.34). The numbers of neutrophils returned to normal levels 18 days after inoculation (Table 7.32).

The number of eosinophils fell significantly on the day of onset of visible parasitaemia (Table 7.35, Fig. 7.10, Appendix Table 24). The mean magnitude of the eosinopaenia was 4,134.6 ± 1,135 mm^2 (Table 7.36).

Significant increases in the number of monocytes occurred seven days after inoculation (Table 7.37, Fig. 7.11, Appendix Table 25); the monocytosis persisted for seven days and had a mean magnitude of 1,520 ± 965 mm^2 (Tables 7.36 and 7.37).

Group D. Similarly, the lambs that were inoculated with the 10^{-1} dilution of TBF-infected blood and then with PI-3 virus at the onset of the TBF parasitaemia showed only a minimal and non-

significant increase in their total leucocyte counts following inoculation (Table 7.38). As before, leucocytosis was brief and was immediately followed by a period of marked and significant leucopaenia (Table 7.38). The mean total leucocyte count decreased from a pre-inoculation level of $9.5 \times 10^9 \pm 2.7 \times 10^9$ per l to a mean nadir of $5.1 \times 10^9 \pm 1.2 \times 10^9$ per l, 8.8 ± 2.8 days after inoculation (Tables 7.38 and 7.39).

The mean lymphocyte count increased significantly on the day after inoculation of TBF-infected blood (Table 7.40). The mean magnitude of the lymphocytosis was 49.4 ± 34.9 mm^2 (Table 7.41). The lymphocytosis was of short duration; the mean lymphocyte count fell precipitously from a pre-inoculation level of $6.6 \times 10^9 \pm 1.9 \times 10^9$ per l to a mean nadir of $2.5 \times 10^9 \pm 0.6 \times 10^9$ per l, 7.2 ± 2.1 days after inoculation (Tables 7.40 and 7.42, Fig. 7.12, Appendix Table 26). The mean magnitude of the lymphocytopaenia was 2,960 ± 1,097.8 mm^2 (Table 7.42).

The mean neutrophil count rose significantly and reached a maximal mean count of $3.2 \times 10^9 \pm 1.0 \times 10^9$ per l, 3.4 ± 1.9 days after inoculation (Tables 7.43 and 7.44). The mean magnitude of the neutrophilia was 550.6 ± 405.5 mm^2 (Table 7.44). The mean neutrophil count then dropped suddenly from a pre-inoculation level of $2.3 \times 10^9 \pm 0.9 \times 10^9$ per l to a mean nadir of $0.8 \times 10^9 \pm 0.2 \times 10^9$ per l, 8.7 ± 1.4 days after the onset of visible parasitaemia (Tables 7.43 and 7.45, Fig. 7.13, Appendix Table 27). The mean magnitude of the neutropaenia was 1,982.3 ± 1,012.5 mm^2 (Table 7.45). The neutrophil counts returned to normal level 18 days after inoculation when the parasitaemia was no longer patent (Table 7.43).

The number of eosinophils decreased significantly on the day following the onset of visible parasitaemia; the eosinophil counts remained depressed for seven days (Table 7.46, Fig. 7.14, Appendix Table 28). The mean magnitude of the eosinopaenia was 3,334 ± 893 mm^2 (Table 7.47).

In contrast, monocytosis occurred early in the reaction and reached significant levels four days after the onset of visible parasitaemia (Table 7.48, Fig. 7.15, Appendix Table 29). The monocytosis persisted for 12 days and had a mean magnitude of 2,870.4 ± 1,655.2 mm^2 (Tables 7.47 and 7.48).

Group E. The mean total leucocyte count in the group inoculated with the 10^{-1} dilution of TBF-infected blood alone increased after inoculation; the increases however were statistically non-significant (Table 7.49). After the initial leucocytosis, the mean total leucocyte count fell from a pre-inoculation level of 10.5 x 10^9 ± 4.8 x 10^9 per l to a mean nadir of 4.7 x 10^9 ± 1.7 x 10^9 per l, 8.0 ± 2.5 days after inoculation (Tables 7.49 and 7.50).

The mean lymphocyte count reflected a similar trend. There was a significant increase in the number of lymphocytes on the day after inoculation, the mean magnitude of the lymphocytosis being 125.3 ± 68.6 mm^2 (Tables 7.51 and 7.52). The lymphocytosis was soon followed by a rapid and significant decline in the number of lymphocytes from a pre-inoculation level of 6.5 x 10^9 ± 2.3 x 10^9 per l to a mean nadir of 2.4 x 10^9 ± 0.8 x 10^9 per l, 7.7 ± 2.7 days after inoculation (Tables 7.51 and 7.53, Figs. 7.4, 7.8, 7.12, Appendix Table 30). The mean magnitude of the lymphocytopaenia

was 2,325.5 ± 1,239.4 mm^2 (Table 7.53).

The number of neutrophils increased initially after inoculation and attained a maximal mean count of 4.8 x 10^9 ± 2.5 x 10^9 per l, 2.6 ± 1.2 days after inoculation (Table 7.54 and 7.55). The mean magnitude of the neutrophilia was 553.1 ± 633.5 mm^2 (Table 7.55). Four days after inoculation, the mean neutrophil count then dropped from a pre-inoculation level of 3.3 x 10^9 ± 2.8 x 10^9 per l to a mean nadir of 0.5 x 10^9 ± 0.3 x 10^9 per l, 8.1 ± 2.2 days after the onset of visible parasitaemia (Tables 7.54 and 7.56, Figs. 7.5, 7.9, 7.13, Appendix Table 31). The mean magnitude of the neutropaenia was 3,323 ± 1,877 mm^2 (Table 7.56).

The eosinophil counts fell markedly during the reaction (Figs. 7.6, 7.10, 7.14, Appendix Table 32); the eosinopaenia lasted from the seventh to the thirteenth day after inoculation and had a mean magnitude of 3,835.2 ± 997.6 mm^2 (Tables 7.57 and 7.58).

On the other hand, the number of monocytes increased during the reaction but the increases were not significant (Table 7.59, Figs. 7.7, 7.11, 7.15, Appendix Table 33). The mean magnitude of the monocytosis was 2,263.3 ± 2,544.5 mm^2 (Table 7.58).

Comparisons. The mean magnitude of the post-inoculation lymphocytosis in the group inoculated with PI-3 virus alone was similar to that of the group infected with TBF alone and to those of the groups with dual infections (Table 7.60). On the other hand, the mean magnitude of the lymphocytopaenia during the reaction was significantly less in the group infected with PI-3 virus alone than in the groups inoculated with TBF alone or in combination with

PI-3 virus (Table 7.60).

The mean magnitude of the post-inoculation neutrophilia was similar in all groups (Table 7.60). The mean magnitude of the neutropaenia was also less in the lambs inoculated with PI-3 virus alone than in the other groups (Table 7.60). The mean magnitude of the neutropaenia in the other groups fell into two overlapping subsets, the greater magnitude being $3,118 \pm 1,410$ mm^2 for the subset containing the group that was inoculated simultaneously with the 10^{-1} dilution of the TBF-infected blood and PI-3 virus and the group infected with TBF alone (Table 7.60).

The mean magnitudes of the eosinopaenia in the five experimental groups were found to be significantly greater in the lambs inoculated with TBF-infected blood alone and in the groups with dual infections than in the group infected with PI-3 virus alone (Table 7.60). Differences between the other groups were not significant (Table 7.60). The eosinopaenia in the group receiving PI-3 virus alone was transient lasting only for one day, whereas, in the other experimental groups the eosinopaenia persisted for seven to nine days and occurred during the overt parasitaemias.

The mean magnitude of the monocytosis was significantly greater in the group that was inoculated with PI-3 virus at the onset of the TBF parasitaemia than in the other experimental groups (Table 7.60). Significant monocytosis only occurred in animals receiving PI-3 virus. The group that received PI-3 virus alone had a monocytosis that persisted for at least three days and occurred 12 days after inoculation. In contrast, the monocytosis in concurrently

infected animals lasted six to seven days and the reaction occurred during the TBF parasitaemia. The monocytosis in the lambs infected with PI-3 virus at the onset of the TBF parasitaemia lasted for at least 12 days and extended beyond the TBF parasitaemia.

Parasitaemia

Group responses. Group E. The lambs infected with TBF alone had a mean prepatent period of 2.8 ± 1.6 days in the range two to seven days (Table 7.61). The incubation period was the same as the prepatent period in 40 percent of the lambs but in 20 percent the visible parasitaemia preceded fever by one day; in the remaining lambs, fever preceded the visible parasitaemia by one to four days (Tables 7.5 and 7.61).

High parasitaemias were observed early in the reaction (Figs. 7.16, 7.17, 7.18). A mean maximal parasitaemia of $10^{9.2 \pm 0.3}$ infected neutrophils per l occurred 1.5 ± 1.2 days after the onset of visible parasitaemia (Table 7.61). The mean duration of the visible parasitaemias was 8.2 ± 1.2 days in the range six to ten days and was similar to the mean duration of the fevers ($t_{18} = 1.824$, ($P > 0.050$) (Tables 7.5 and 7.61). The mean magnitude of the parasitaemia was 17,733.7 ± 2,338 mm^2 (Table 7.61).

Group B. In the group inoculated simultaneously with the 10^{-1} dilution of TBF-infected blood and PI-3 virus, the prepatent period always lasted two to three days after inoculation, the mean being 2.4 ± 0.5 days (Table 7.62). The prepatent period in 60 percent of the lambs was the same as the incubation period but in 20 percent, visible parasitaemia preceded fever by no more than one day and vice versa in

the rest of the lambs (Tables 7.2 and 7.62).

High parasitaemias were recorded early in the reaction (Fig. 7.16). A mean maximal parasitaemia of $10^{9.3 \pm 0.2}$ infected neutrophils per l was observed 1.3 ± 1.2 days after the onset of visible parasitaemia which persisted for 9.0 ± 1.4 days in the range seven to 11 days (Table 7.62). The mean duration of the visible parasitaemia was significantly longer than the mean duration of the fevers ($t_{18} = 2.572$, $P < 0.020$) (Tables 7.2 and 7.62). The mean magnitude of the parasitaemia was 17,776.7 ± 7,005.6 mm^2 (Table 7.62).

Group C. The prepatent period in the lambs that were inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus ranged from three to eight days the mean being 4.0 ± 1.7 days (Table 7.63). In 30 percent of the lambs, the incubation period was the same as the prepatent period; in 70 percent the fever preceded the visible parasitaemia by one to three days (Tables 7.3 and 7.63).

During the reaction, high parasitaemias were recorded (Fig. 7.17). A mean maximal parasitaemia of $10^{9.1 \pm 0.1}$ infected neutrophils per l occurred 1.9 ± 1.5 days after the onset of visible parasitaemia (Table 7.63). The mean duration of the visible parasitaemias was 8.6 ± 1.3 days in the range six to ten days and was similar to the mean duration of the fevers ($t_{18} = 0.148$, $P > 0.050$) (Tables 7.3 and 7.63). The mean magnitude of the parasitaemia was 18,784 ± 2,697 mm^2 (Table 7.63).

Group D. The lambs that were inoculated with the 10^{-1} dilution of TBF-infected blood and with PI-3 virus at the onset of the

TBF parasitaemia were parasitaemic two to three days after inoculation, the mean prepatent period being 2.2 ± 0.4 days (Table 7.64). The incubation period was the same as the prepatent period in 80 percent of the lambs; in the remaining lambs, the visible parasitaemia preceded fever by one day (Tables 7.4 and 7.64).

Likewise, high parasitaemias occurred early in the reaction (Fig. 7.18). The group had a mean maximal parasitaemia of $10^{9.2 \pm 0.1}$ infected neutrophils per l, 1.3 ± 1.0 days after the onset of visible parasitaemia which lasted for 9.7 ± 2.2 days in the range seven to 15 days (Table 7.64). The mean duration of the febrile responses was similar to the mean duration of the visible parasitaemias ($t_{18} = 0.445$, $P > 0.050$) (Tables 7.4 and 7.64). The mean magnitude of the parasitaemia was 20,886 ± 4,669 mm^2 (Table 7.64).

Comparisons. The mean prepatent period was significantly longer in the lambs that were inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus than in the groups inoculated with the 10^{-1} dilution of TBF-infected blood alone or in combination with PI-3 virus (Table 7.65).

The differences in the mean maximal parasitaemias, the mean durations and magnitudes of the visible parasitaemias between the experimental groups were minimal and non-significant. Maximum parasitaemias occurred at the same time in all groups after the onset of visible parasitaemia.

Virus excretion

Group response. Group A. In the lambs that were inoculated intranasally with PI-3 virus alone, the virus was isolated from the

nasal swabs in nine out of ten lambs three days after inoculation and from the tenth lamb on day four (Table 7.66). Thirty percent of the lambs excreted the virus one to two days before the onset of fever; 60 percent yielded the virus on the day of the onset of fever and the remaining lamb was positive for PI-3 virus two days after the onset of the febrile reaction. The mean duration of the virus excretion was 3.7 ± 1.0 days in the range three to five days (Table 7.67).

Group B. Seven out of the ten lambs that were inoculated simultaneously with the 10^{-1} dilution of TBF-infected blood and PI-3 virus were positive for PI-3 virus three days after inoculation; the three other lambs yielded the virus on day four (Table 7.66). Parainfluenza-3 virus was recovered from 20 percent of the lambs two days before the onset of fever and from 70 and ten percent on the day of the onset of fever and two days after the onset of fever, respectively. The mean duration of virus excretion was 5.9 ± 1.8 days in the range four to eight days (Table 7.67).

Group C. Parainfluenza-3 virus was likewise re-isolated from seven out of the ten lambs inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus three days after inoculation and from the three other lambs on day four (Table 7.66). Thirty percent of the lambs shed the virus one to two days before the onset of fever whilst nasal swabs from 60 percent were positive for PI-3 virus on the day of the onset of fever; the remaining lamb excreted the virus two days after the onset of the febrile reaction. The virus excretion time ranged from four to eight days

the mean duration being 5.7 ± 1.5 days (Table 7.67).

Group D. In the group that was inoculated with PI-3 virus at the onset of the TBF parasitaemia, excretion of the virus occurred early; the virus was recovered from three out of ten lambs two days after inoculation and from the rest on day three (Table 7.66). The mean duration of virus excretion was 8.5 ± 1.4 days in the range six to ten days (Table 7.67). The virus was also isolated from the lungs of one of the two lambs that died in this experimental group.

Comparisons. Differences between the mean durations of virus excretion were significant ($F^{3}_{36} = 19.50$; $P < 0.010$). The mean duration of virus excretion was significantly shorter in the lambs inoculated with PI-3 virus alone than in the groups with dual infections (Table 7.68). In contrast, the mean duration of virus excretion was found to be significantly longer in the lambs that were exposed to the virus at the onset of the TBF parasitaemia than in the groups inoculated simultaneously with both agents.

Antibody assays

Group responses. Neither HI nor VN antibodies to PI-3 virus were detected in the sera collected immediately before virus inoculation.

Group A. The lambs that were inoculated with PI-3 virus alone developed high titres of HI antibodies following inoculation. The mean HI titre of 3.9 ± 0.8 was detected seven days after exposure to the virus; this rose significantly to 5.7 ± 0.9 on day 14 and to

7.3 ± 0.8 on day 21 ($t_9 = 7.229$, $P < 0.001$ and $t_9 = 7.239$, $P < 0.001$, respectively). Virus neutralising antibodies were also demonstrated in the pooled sera; the antibody titre for the group was 3.7 ± 0.2 on day seven; this continued to rise reaching 5.6 ± 0.3 on day 14 and 7.2 ± 0.2 on day 21.

Group B. The group of lambs that were inoculated simultaneously with the 10^{-1} dilution of the TBF-infected blood and PI-3 virus also had rising HI antibody titres to PI-3 virus, the mean titre being 2.2 ± 0.8 on day seven. The mean HI antibody titre rose significantly to 3.9 ± 1.0 on day 14 and to 4.8 ± 1.1 on day 21 ($t_9 = 4.644$, $P < 0.010$ and $t_9 = 2.866$, $P < 0.020$, respectively). Virus neutralising antibodies were found in the pooled serum samples; the VN antibody titre for the group was 3.4 ± 0.3 on day seven, 4.6 ± 0.3 on day 14 and 5.0 ± 0.3, 21 days after virus inoculation.

Group C. The group that was inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus had a mean serum HI antibody titre of 2.4 ± 0.8 seven days after inoculation; this increased significantly to 4.6 ± 0.9 on day 14 ($t_9 = 13.533$, $P < 0.001$). The rise in the mean HI titre to 5.0 ± 1.6, 21 days after inoculation was minimal and non-significant ($t_9 = 0.803$, $P > 0.050$). The virus neutralising antibody titre for the group was 2.7 ± 0.2 on day seven, 4.2 ± 0.3 on day 14 and 4.8 ± 0.3 on day 21.

Group D. The lambs that were inoculated with the 10^{-1} dilution of the TBF-infected blood and with PI-3 virus at the onset of the TBF parasitaemia similarly developed HI antibodies to PI-3 virus but the rises in the titres were not significant ($t_8 = 1.590$,

$P > 0.050$; $t_7 = 1.785$, $P < 0.10$); the mean HI antibody titre was 2.8 ± 0.7 on day seven, 3.3 ± 0.9 on day 14 and 4.3 ± 0.9 on day 21. The group had a VN antibody titre of 2.6 ± 0.2 on day seven; this increased to 4.1 ± 0.3 on day 14 but was unchanged at 4.3 ± 0.2, 21 days after inoculation.

Comparisons. The mean HI antibody titres to PI-3 virus in the lambs infected with the virus alone were consistently and significantly higher than the mean HI titres in the groups with dual infections (Table 7.69). Similarly, the VN antibody titres in the group infected with PI-3 virus alone were higher than those of the groups infected with both agents but the differences were significant only on day 21 (Table 7.70).

DISCUSSION

Parainfluenza-3 virus alone reactions. The first recorded isolation of an ovine strain of PI-3 virus was made by Hore in 1966 from lambs in an intensively run flock in which there had been an outbreak of mild respiratory disease. The strain was later used by Hore and Stevenson (1969) in the experimental production of an upper respiratory tract infection and pneumonia in lambs. In the experimental infection, the onset of illness was marked by a rise in temperature and the appearance of mucoid nasal discharges two to three days after inoculation. Other clinical signs were minimal.

Hore, Stevenson, Gilmour, Vantsis and Thompson (1968) isolated PI-3 virus from a group of ten newly introduced lambs involved

in an outbreak of respiratory disease and described the clinical course and pathological changes of the natural infection. Illness was first manifested by temperature reactions exceeding 40.6°C which occurred 11 days after the introduction of the lambs. Temperatures ranging from 40.7°C to 41.8°C were recorded during the febrile reaction which lasted for at least four days. Apart from elevated temperatures and the presence of slight purulent nasal discharges, no other clinical abnormalities were observed in eight lambs. One lamb developed more obvious signs of respiratory disease and was dyspnoeic, dull and anoretic for four days; the other lamb remained clinically normal throughout the period of observation.

Biberstein and his colleagues (1971) investigated the clinical, microbiological and pathological responses of conventionally reared lambs to infection with PI-3 virus; their findings added nothing new to the observations of Hore and Stevenson (1969). They found that virus inoculation alone resulted in almost imperceptible illness.

My findings were similar; the intranasal inoculation of PI-3 virus alone induced a low-grade febrile response and transient respiratory signs in the lambs.

Haematological changes attributable to PI-3 virus infection in sheep have not been reported. However, in calves inoculated with PI-3 virus, Woods and his co-workers (1964) found that the total leucocyte counts were within the normal ranges; they however noted a moderate lymphocytosis on days six to 17. Hamdy and his colleagues (1963) observed leucopaenia in most calves exposed to

PI-3 virus two to four days later. Leucopaenia or a trend in this direction was also noted by Saunders and Berman (1964) in some calves two to three days after exposure to the virus

I likewise found the changes in the total leucocyte counts in the lambs infected with PI-3 virus alone to be minimal. The mean lymphocyte count increased after virus inoculation but the lymphocytosis was not significant. A moderate lymphocytopaenia was noted 6.5 ± 3.1 days after the virus inoculation, the lymphocyte counts returning to normal levels on day 12. There was also a non-significant neutrophilia following inoculation; during the febrile reaction the neutrophil counts were within normal ranges. The number of eosinophils decreased after virus exposure but the eosinopaenia was significant only on day six. Early in the reaction, changes in the monocyte counts were slight. Subsequently, the number of monocytes increased reaching significant levels 12 to 14 days after virus inoculation.

Hore and Stevenson (1969) recovered PI-3 virus from the nasal passages of five out of ten lambs 24 hours after inoculation and thereafter from each lamb until day six, from six out of seven lambs on day seven and from four out of five lambs on day eight. Similarly, other workers have recovered PI-3 virus from nasal swabs taken between the first to the seventh day of exposure (Biberstein et al., 1971; Sharp et al., 1978).

In the present study, PI-3 virus was re-isolated from the nasal swabs in nine out of ten lambs inoculated with PI-3 virus alone on day three and from the tenth lamb on day four. The duration

of virus excretion ranged from three to five days and isolations from the nasal secretions were regularly achieved during the height of the febrile response.

In naturally infected lambs, Hore and his colleagues (1968) detected HI antibodies to PI-3 virus at the time of or immediately following the febrile response. Serologic responses to experimental PI-3 virus infection were often detected six to seven days after inoculation (Woods, 1968; Hore and Stevenson, 1969; Smith, 1975). Serum HI antibody titres of 4.3 or greater were found by Howe, Woods and Marquis (1966) in serum samples from Bighorn sheep. Hore (1969) on his study of 500 sera from sheep recorded a mean HI titre of 6.3 Karrar (1977) examined 100 serum samples from sheep of different origins for HI antibodies for PI-3 virus and obtained titres ranging from two to seven. Virus neutralising antibody titres of 4.3 to 6.6 per 0.1 ml were found by Singh and Ata (1967) in sheep sera. Smith (1975) demonstrated serum neutralising antibody to PI-3 virus in sheep seven days after inoculation at a mean titre of one; the mean titre at three weeks was 4.3. Hoerlein and his co-workers (1959) demonstrated that sera with an HI titre of 4.3 or greater to PI-3 virus always had neutralising capacity in tissue culture. The HI and VN antibody titres observed in my study were in accordance.

Tick-borne fever alone reactions. The clinical responses of the lambs inoculated with the TBF-infected blood alone were similar to those observed in the group of eight sheep that were used originally to assay the clinical, haematological and parasitological

parameters of tick-borne fever (Chapter Three) (Table 7.71). Likewise, the haematological features (Table 7.72) and the parameters of the parasitaemia (Table 7.71) were similar to those of the original group of eight sheep.

Dual infections. Sharp and his colleagues (1978) reported the development of severe clinical disease and pneumonia in a high proportion of lambs inoculated with PI-3 virus in combination with P. haemolytica but not in lambs inoculated with either agents alone. Concurrent infection with TBF and PI-3 virus in my experiments produced similar results in lambs. The groups inoculated simultaneously with both agents had higher fevers and showed respiratory distress. The severity of the clinical reactions was found to be even greater in the lambs infected with TBF and later exposed to PI-3 virus at the onset of the TBF parasitaemia; 20 percent of these lambs died. The mean duration of the fevers was significantly longer in the lambs infected with TBF alone and in the lambs with dual infections than in the lambs exposed to the virus alone. Analysis of the magnitudes of the fevers confirmed that the febrile responses in the lambs receiving the virus alone were significantly lower than the responses in the other groups.

The contrast between the groups with dual infections and the group infected with TBF alone was less distinct. Nevertheless, the mean magnitudes of the fevers fell into three overlapping subsets, the greater subset being the group inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus and the group that was exposed to the virus at the onset of TBF parasitaemia.

Pasteurella haemolytica was isolated from the lungs of two of the dual-infected lambs that died. Histological examination of the lung sections revealed the presence of typical exudative Pasteurella pneumonia lesions and lesions of proliferative nature generally regarded as characteristic of PI-3 virus infection (Hore et al., 1968). These results together with the findings reported in Chapter Six in which it was shown that TBF affected the nasal carriage of P. haemolytica in sheep the rate of isolation of the bacterium being increased during the TBF parasitaemia, are consistent with the association of tick-borne fever with outbreaks of pneumonia in sheep postulated by Foggie (1951) and Øveras (1972).

The haematological responses in the lambs infected with TBF alone or in combination with PI-3 virus were in marked contrast to the haematological responses of the lambs inoculated with PI-3 virus alone. The magnitudes of the lymphocytopaenia and the neutropaenia during the reactions were significantly less in the lambs infected with the virus alone than in the other groups. The mean magnitude of the neutropaenias in the other groups fell into two subsets, the greater subset being the group inoculated simultaneously with the 10^{-1} dilution of TBF-infected blood and PI-3 virus and the group infected with TBF alone.

Profound eosinopaenias occurred during the patent parasitaemias in the groups with dual infections and the group infected with TBF alone; the eosinopaenia in the lambs receiving PI-3 virus alone was brief, lasting only for one day.

Significant increases in the number of monocytes occurred in the groups that were inoculated with the virus alone or in combination with TBF but not in the group infected with TBF alone. The mean magnitude of the monocytosis was significantly greater in the lambs that were exposed to PI-3 virus at the onset of the TBF parasitaemia than in the other groups.

Concurrent infection with PI-3 virus had no effect on the course and severity of the TBF parasitaemia. The mean durations and magnitudes of the visible parasitaemias, the maximal parasitaemias and day on which maximal parasitaemias occurred were not significantly different between the groups with dual infections and the group infected with TBF alone. The mean prepatent period was significantly longer in the group that was inoculated simultaneously with the 10^{-3} dilution of TBF-infected blood and PI-3 virus than in the groups given the 10^{-1} dilution of TBF-infected blood alone or in combination with the PI-3 virus. This finding was in agreement with the observation made by Snodgrass (1974) that the prepatent period varied inversely with the dose of TBF-infected blood.

On the other hand, concurrent infection with TBF affected the virus excretion times. The mean duration of virus excretion was significantly longer in the lambs with dual infections than in the lambs infected with the virus alone. Differences between the mean durations of virus excretion in the groups infected with both agents were also significant; the lambs that were inoculated with the virus at the onset of the TBF parasitaemia excreted PI-3 virus

for a longer period than the lambs inoculated simultaneously with TBF and PI-3 virus. The concurrently infected animals shed the virus for three to eight days whereas, the lambs exposed to PI-3 at the onset of the TBF parasitaemia excreted the virus for six to ten days. Virus was also isolated from the lungs of one of the two lambs that died in this experimental group.

Virus potentiation but not rickettsial potentiation occurred in the dual-infected animals as evidenced by the prolonged period during which PI-3 virus was isolated from the nasal swabs. The exacerbation of the respiratory disease and prolongation of the virus excretion were associated with a marked depression in the production of antiviral antibodies such that virus infection was allowed to persist beyond the three to five days period observed in the lambs infected with PI-3 virus alone. A possible explanation relates the antibody levels to virus excretion and clinical disease because my lambs that were inoculated simultaneously with TBF and PI-3 virus likewise developed serum HI and VN antibodies to PI-3 virus but the titres were lower than the titres in the lambs infected with the virus alone; the antibody responses were found to be even less in the lambs infected with TBF and later exposed to PI-3 virus at the onset of the TBF parasitaemia. Such an inverse relationship had been noted by other workers. Smith (1975) for example failed to recover PI-3 virus from lambs with pre-challenge serum VN antibody titres to PI-3 virus of six or greater but did re-isolate the virus for four to seven days from lambs with pre-challenge titres of two or less. Gilmour and his group (1968) also

did not recover PI-3 virus from sheep with pre-challenge serum HI titres of five or greater but recovered the virus from sheep with HI titres of four or less for five days.

Virus potentiation has been found consistently in studies of flavivirus infections in immunosuppressed animals (Zlotnik, Smith, Grant and Peacock, 1970; Camenga, Nathanson and Cole, 1974; Bhatt and Jacoby, 1976). Camenga and his colleagues, for example, showed that a single dose of cyclophosphamide given 24 hours after virus inoculation, converted an inapparent West Nile virus infection into a lethal encephalitis in mice. The cyclophosphamide-potentiated infection was characterised by suppressed response of neutralising antibody, prolonged viremia, enhanced titres of virus in the brain, severe encephalitis and high mortality. They further demonstrated that neutralising antibody played a critical role in recovery from the potentially fatal encephalitis; transfer of immune serum or syngeneic spleen cells depleted of T-lymphocytes from mice immune to West Nile virus produced a dramatic reversal of the cyclophosphamide-induced potentiation of West Nile virus infection. An apparent potentiation of louping-ill virus infection had been reported by Buxton and his co-workers (1980) in mice infected with Toxoplasma gondii. They found that when T. gondii was given to a group of mice seven or 14 days before louping-ill virus there was more intense viral replication, a delayed and reduced antibody response to the virus, and more severe clinical reactions in the animals with dual infections than in the mice given the virus alone. Similar alterations in the course of louping-ill virus infection were observed by Reid and his colleagues (1979a) in mice with chronic

Trypanosoma brucei infection.

These results support the hypothesis that infection with *C. phagocytophila* induces a significant degree of immunosuppression.

Table 7.1 Clinical parameters in ten lambs inoculated with parainfluenza-3 virus

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
991	3	40.7	0	3	875
996	3	40.7	1	3	875
61	2	41.3	4	5	1,250
62	3	40.9	1	3	1,100
87	2	40.8	1	5	850
88	2	40.9	1	3	1,225
93	2	40.8	1	4	1,125
94	2	41.0	1	5	1,575
107	2	41.8	1	5	1,350
108	2	41.3	1	4	1,275
mean	2.3	41.0	1.2	4.0	1,150
standard deviation	0.4	0.3	1.0	0.9	234

Table 7.2 Clinical parameters in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
133	3	42.0	1	6	2,200
134	4	42.0	0	6	3,038
149	2	42.0	1	7	2,225
150	2	42.1	1	7	1,900
151	2	42.3	0	8	4,250
988	3	41.7	0	9	2,375
994	3	41.9	1	6	1,975
63	2	41.6	3	9	2,575
89	1	41.5	3	9	2,625
90	2	41.8	4	8	4,425
mean	2.4	41.9	1.4	7.5	2,758
standard deviation	0.8	0.2	1.4	1.2	896

Table 7.3 Clinical parameters in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
135	2	41.9	2	9	2,775
137	2	41.9	0	10	3,875
152	3	42.3	3	11	4,725
153	3	42.5	1	10	3,525
154	2	42.2	1	8	3,475
981	5	41.6	1	5	1,550
993	5	42.0	4	7	3,500
64	2	41.5	1	9	2,700
91	3	41.8	3	9	3,950
92	3	41.7	2	7	2,875
mean	3.0	41.9	1.8	8.5	3,295
standard deviation	1.1	0.3	1.2	1.7	868

Table 7.4 Clinical parameters in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
139	3	42.2	0	11	3,950
140	3	41.9	2	8	4,650
163	3	42.4	0	7	3,350
164	2	42.3	1	6	2,375
95	2	42.0	1	12	4,625
96	2	41.7	1	10	3,675
109	2	42.1	2	10	3,350
113	2	42.2	2	11	4,200
115	3	41.9	0	9	3,400
116	3	41.9	1	9	3,400
mean	2.5	42.0	1.0	9.3	3,698
standard deviation	0.5	0.2	0.8	1.8	687

Table 7.5 Clinical parameters in ten lambs inoculated with TBF-infected blood 10^{-1}

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of fever (mm^2)
68	1	41.4	1	4	1,625
984	2	41.8	1	5	1,850
990	1	41.9	4	8	2,100
78	2	41.5	2	5	1,925
79	3	42.1	0	6	2,150
992	2	41.5	4	12	3,125
797	3	42.2	0	4	1,375
62	2	41.8	1	9	3,175
101	2	41.7	2	5	1,525
103	2	41.9	2	8	2,225
mean	2.0	41.7	1.7	6.6	2,108
standard deviation	0.6	0.2	1.4	2.5	614

Table 7.6 Clinical parameters: significant subsets of the experimental groups

Parameter	Variance ratio (d.f. 4, 45)	Subsets significant at the 1% level of probability
Incubation period	4.43**	E A B D C
Maximal temperature	21.59**	A / E B C D
Day of occurrence of maximal temperature	0.77	D A B E C
Duration of fever	13.16**	A / E B C D
Magnitude of fever	20.77**	A / E B / C / D

** $P < 0.010$

A = Group inoculated with PI-3 virus alone

B = Group inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

C = Group inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

D = Group inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia

E = Group inoculated with TBF-infected blood 10^{-1} alone

Table 7.7 Means of the daily total leucocyte counts of ten lambs inoculated with PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Mean and standard deviation of total leucocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	9.2 ± 1.1	
1	9.9 ± 1.4	-0.3 ± 0.3
2	9.4 ± 1.4	0.1 ± 0.4
3	9.5 ± 1.4	0.07 ± 0.4
4	9.5 ± 1.2	0.1 ± 0.3
5	9.1 ± 1.3	0.5 ± 0.4
6	9.4 ± 1.2	0.1 ± 0.3
7	9.1 ± 1.6	0.4 ± 0.6
8	9.0 ± 1.6	0.4 ± 0.5
9	9.0 ± 1.5	0.3 ± 0.5
10	9.1 ± 1.4	0.5 ± 0.5
11	9.4 ± 1.5	0.1 ± 0.5
12	9.9 ± 1.9	-0.3 ± 0.5
13	10.0 ± 1.9	-0.4 ± 0.5
14	10.0 ± 1.4	-0.4 ± 0.4

Table 7.8 Magnitudes of the post-inoculation lymphocytosis in ten lambs inoculated with PI-3 virus alone

Sheep No.	Magnitude of the lymphocytosis (mm^2)
991	257.0
996	15.0
61	12.0
62	101.5
87	1.5
88	327.5
93	343.0
94	108.0
107	17.5
108	233.5
mean	141.6
standard deviation	136.4

Table 7.9 Means of the daily lymphocyte counts of ten lambs inoculated with PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Mean and standard deviation of lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	6.1 ± 0.7	
1	6.8 ± 0.9	-0.6 ± 0.3
2	6.3 ± 1.0	-0.2 ± 0.2
3	6.2 ± 0.7	-0.1 ± 0.2
4	6.4 ± 0.9	-0.2 ± 0.2
5	6.2 ± 0.7	-0.1 ± 0.2
6	6.3 ± 0.5	-0.2 ± 0.2
7	6.2 ± 0.5	-0.01 ± 0.3
8	5.6 ± 1.0	0.5 ± 0.4
9	5.8 ± 0.9	0.3 ± 0.3
10	5.9 ± 1.4	0.5 ± 0.5
11	5.9 ± 0.9	0.2 ± 0.3
12	6.4 ± 0.9	-0.2 ± 0.3
13	6.6 ± 1.0	-0.5 ± 0.3
14	6.7 ± 1.0	-0.5 ± 0.3

Table 7.10 Lymphocyte count nadirs and magnitudes of the lymphocytopaenia in ten lambs inoculated with PI-3 virus alone

Sheep No.	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Magnitude of the lymphocytopaenia (mm^2)
991	5.6	6	205.0
996	4.9	2	416.0
61	2.4	10	1,274.0
62	4.8	10	436.5
87	5.2	3	62.0
88	5.2	8	310.0
93	3.9	9	341.0
94	5.0	9	370.0
107	5.3	3	206.0
108	5.7	5	100.5
mean	4.8	6.5	372.1
standard deviation	0.9	3.1	341.5

Table 7.11 Means of the daily neutrophil counts of ten lambs inoculated with PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Mean and standard deviation of total leucocyte counts ($\times 10^9/l$)	Mean and standard error of difference from pre-inoculation count ($\times 10^9/l$)
0	2.6 ± 0.9	
1	2.8 ± 0.8	-0.2 ± 0.2
2	2.7 ± 0.8	-0.1 ± 0.2
3	2.6 ± 1.0	-0.04 ± 0.1
4	2.7 ± 1.0	-0.1 ± 0.1
5	2.6 ± 0.8	0.0 ± 0.1
6	2.7 ± 1.1	-0.1 ± 0.1
7	2.7 ± 1.0	-0.1 ± 0.1
8	3.0 ± 1.7	-0.4 ± 0.3
9	2.9 ± 1.4	-0.1 ± 0.2
10	2.8 ± 1.6	-0.3 ± 0.4
11	2.5 ± 1.1	0.03 ± 0.2
12	2.7 ± 1.1	0.02 ± 0.1
13	2.7 ± 0.9	-0.09 ± 0.1
14	2.7 ± 0.9	-0.1 ± 0.1

Table 7.12 Magnitudes of the post-inoculation neutrophilia and the neutropaenia in ten lambs inoculated with PI-3 virus alone

Sheep No.	neutrophilia (mm^2)	neutropaenia (mm^2)
991	121.0	352.0
996	369.0	75.0
61	465.0	98.5
62	1.0	175.0
87	10.1	175.0
88	781.0	738.0
93	862.5	100.0
94	11.5	893.5
107	92.0	587.0
108	30.0	906.5
mean	241.5	410.0
standard deviation	323.7	339.6

Table 7.13 Medians of the daily eosinophil counts of ten lambs inoculated with parainfluenza-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Medians of the eosinophil counts ($x10^9/l$)	Means and standard error of difference from pre-inoculation count ($x10^9/l$)
0	0.251	
1	0.260	0.01 ± 0.03
2	0.193	0.02 ± 0.03
3	0.225	0.02 ± 0.03
4	0.205	0.03 ± 0.03
5	0.186	0.08 ± 0.04
6	0.131	0.08 ± 0.03*
7	0.146	0.05 ± 0.03
8	0.173	0.07 ± 0.04
9	0.235	0.09 ± 0.05
10	0.251	0.02 ± 0.04
11	0.255	0.03 ± 0.06
12	0.247	0.01 ± 0.03
13	0.275	-0.01 ± 0.01
14	0.305	-0.04 ± 0.02

* $P < 0.050$

Table 7.14 Magnitudes of the eosinopaenia and monocytosis in ten lambs inoculated with PI-3 virus alone

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
991	1,069	242
996	2,157	147
61	2,133	181
62	2,236	82
87	853	685
88	1,058	561
93	2,590	487
94	59	621
107	1,189	87
108	771	380
Mean	1,411.4	347.3
Standard deviation	815.5	229.0

Table 7.15 Medians of the daily monocyte counts of ten lambs inoculated with PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Medians of the monocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	0.229	
1	0.232	0.03 ± 0.02
2	0.235	0.01 ± 0.02
3	0.225	0.01 ± 0.03
4	0.230	0.04 ± 0.02
5	0.200	0.01 ± 0.03
6	0.239	-0.01 ± 0.01
7	0.229	-0.003 ± 0.01
8	0.250	-0.04 ± 0.04
9	0.228	-0.01 ± 0.01
10	0.246	-0.02 ± 0.01
11	0.243	-0.02 ± 0.01
12	0.246	-0.01 ± 0.003**
13	0.232	-0.01 ± 0.003**
14	0.234	-0.02 ± 0.008*

* $P < 0.050$

** $P < 0.010$

Table 7.16 Means of the daily total leucocyte counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of total leucocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	10.3 ± 1.4	
1	-	10.8 ± 1.4	-0.2 ± 0.09
2	-	10.5 ± 1.8	-0.2 ± 0.3
3	+	10.2 ± 2.0	0.6 ± 0.4
4	+	7.6 ± 2.3	2.9 ± 0.8**
5	+	7.5 ± 1.8	2.7 ± 0.6**
6	+	6.5 ± 1.5	3.0 ± 0.9*
7	+	5.6 ± 1.1	4.6 ± 0.4***
8	+	5.9 ± 1.2	4.5 ± 0.5***
9	+	5.7 ± 1.2	4.5 ± 0.5***
10	+	5.6 ± 0.8	4.6 ± 0.4***
11	+	5.8 ± 1.5	4.1 ± 0.5***
12	+	5.7 ± 1.4	4.5 ± 0.4***
13	+	6.1 ± 1.5	4.3 ± 0.4***
14	-	5.8 ± 1.7	4.6 ± 0.5***
15	-	6.1 ± 1.8	4.2 ± 1.9
16	-	6.5 ± 1.9	3.7 ± 1.8
17	-	7.2 ± 2.3	3.2 ± 2.4
18	-	7.9 ± 2.4	2.6 ± 0.7

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.17 Total leucocyte count nadirs in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Total leucocyte count nadir ($x10^9/l$)	Days after inoculation
133	3.6	14
134	4.0	14
149	4.9	10
150	4.5	8
151	5.3	14
988	4.3	9
994	3.8	6
63	5.7	6
89	6.3	13
90	4.7	7
mean	4.7	10.1
standard deviation	0.8	3.3

Table 7.18 Magnitudes of the post-inoculation lymphocytosis in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	magnitude of the lymphocytosis (mm^2)
133	60.5
134	30.5
149	211.5
150	5.0
151	4.0
988	2.0
994	33.5
63	79.5
89	5.0
90	4.0
mean	43.5
standard deviation	64.8

Table 7.19 Means of the daily lymphocyte counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	7.0 ± 0.9	
1	-	7.2 ± 0.8	-0.2 ± 0.08*
2	-	6.7 ± 0.9	0.2 ± 0.3
3	+	5.6 ± 1.8	1.4 ± 0.5*
4	+	4.0 ± 1.6	3.1 ± 0.5***
5	+	3.6 ± 1.0	3.4 ± 0.3***
6	+	3.0 ± 0.9	4.0 ± 0.3***
7	+	2.7 ± 0.7	4.3 ± 0.3***
8	+	3.3 ± 0.3	3.7 ± 0.3***
9	+	3.2 ± 0.3	3.7 ± 0.3***
10	+	3.8 ±+0.4	3.1 ± 0.3***
11	+	4.0 ± 0.7	3.1 ± 0.2***
12	+	4.4 ± 1.0	2.6 ± 0.3***
13	+	4.5 ± 1.3	2.5 ± 0.3***
14	-	4.5 ± 1.1	2.5 ± 0.3***
15	-	4.6 ± 1.5	2.4 ± 0.5***
16	-	4.9 ± 1.4	2.1 ± 0.4**
17	-	5.0 ± 1.6	1.9 ± 0.5**
18	-	5.3 ± 1.5	1.7 ± 0.5**

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.20 Lymphocyte count nadirs and magnitudes of the lymphocytopaenia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Magnitude of the lymphocytopaenia (mm^2)
133	2.1	6	3,928
134	1.8	7	3,484
149	1.5	4	2,691
150	1.7	4	2,781
151	2.7	6	4,460
988	2.6	7	4,045
994	2.1	6	3,087
63	2.5	7	2,896
89	3.6	8	2,786
90	2.0	7	3,531
mean	2.3	6.2	3,368.9
standard deviation	0.6	1.3	618.9

Table 7.21 Means of the daily neutrophil counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of neutrophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	2.6 ± 0.5	
1	-	3.2 ± 0.8	-0.5 ± 0.2*
2	-	3.1 ± 1.0	-0.4 ± 0.2
3	+	4.1 ± 1.7	-1.4 ± 0.4*
4	+	3.1 ± 0.9	-0.5 ± 0.2
5	+	3.3 ± 0.9	-0.7 ± 0.3
6	+	2.8 ± 1.1	0.3 ± 0.1
7	+	2.2 ± 0.9	1.1 ± 0.3*
8	+	1.9 ± 1.0	1.4 ± 0.3**
9	+	1.9 ± 1.0	1.4 ± 0.3***
10	+	1.3 ± 0.8	1.2 ± 0.3**
11	+	1.2 ± 0.9	1.4 ± 0.2***
12	+	0.9 ± 0.5	1.6 ± 0.1***
13	+	1.2 ± 0.9	1.5 ± 0.1***
14	-	0.9 ± 0.4	1.6 ± 0.2***
15	-	1.2 ± 0.6	1.4 ± 0.1***
16	-	1.3 ± 0.6	1.3 ± 0.2***
17	-	1.6 ± 0.8	1.0 ± 0.2***
18	-	2.0 ± 1.0	0.6 ± 0.2

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.22 Maximal neutrophil counts and magnitudes of the neutrophilia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Maximal neutrophil count ($x10^9/l$)	Days after inoculation	Magnitude of the neutrophilia (mm^2)
133	4.8	5	525.0
134	5.1	3	1,054.0
149	3.9	3	269.5
150	5.9	3	825.5
151	6.3	3	1,272.0
988	4.0	5	511.5
994	2.4	2	143.0
63	7.0	3	293.0
89	3.6	5	1,141.5
90	3.7	3	383.0
mean	4.7	3.5	641.8
standard deviation	1.4	1.0	402.3

Table 7.23 Neutrophil count nadirs and magnitudes of the neutropaenia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia	Magnitude of the neutropaenia (mm^2)
133	0.5	7	3,917.2
134	0.5	10	2,305.0
149	0.5	9	3,786.5
150	0.5	9	3,220.5
151	0.6	13	2,329.0
988	0.4	6	3,576.0
994	0.7	6	3,109.2
63	1.2	6	2,775.0
89	1.0	11	1,458.0
90	1.2	7	2,660.5
mean	0.7	8.4	2,913.7
standard deviation	0.2	2.4	764.1

Table 7.24 Medians of the daily eosinophil counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus and signifiance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the eosinophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.476	
1	-	0.370	0.2 ± 0.1
2	-	0.308	0.07 ± 0.05
3	+	0.161	0.2 ± 0.1
4	+	0.141	0.4 ± 0.1**
5	+	0.051	0.4 ± 0.1**
6	+	0.031	0.4 ± 0.1**
7	+	0.095	0.4 ± 0.1**
8	+	0.083	0.4 ± 0.1*
9	+	0.040	0.4 ± 0.1**
10	+	0.154	0.4 ± 0.1*
11	+	0.142	0.4 ± 0.1*
12	+	0.105	0.4 ± 0.1*
13	+	0.102	0.3 ± 0.2
14	-	0.175	0.3 ± 0.2
15	-	0.142	0.3 ± 0.2
16	-	0.237	0.3 ± 0.1
17	-	0.220	0.2 ± 0.1
18	-	0.251	0.1 ± 0.09

* $P < 0.050$

** $P < 0.010$

Table 7.25 Magnitudes of the eosinopaenia and monocytosis in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
133	4,072	1,933
134	4,118	71
149	4,728	245
150	6,191	2,084
151	5,228	2,748
988	3,560	2,744
994	4,124	2,192
63	3,399	2,657
89	2,602	3,324
90	3,679	2,435
Mean	4,170	2,043.3
Standard deviation	1,011	1,070.7

Table 7.26 Medians of the daily monocyte counts of ten lambs inoculated simultaneously with TBF-10^{-1} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the monocyte counts ($x10^9/l$)	Means and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.224	
1	-	0.203	0.06 ± 0.03
2	-	0.237	0.01 ± 0.01
3	+	0.261	-0.01 ± 0.01
4	+	0.328	-0.01 ± 0.03
5	+	0.309	-0.03 ± 0.03
6	+	0.379	-0.09 ± 0.05
7	+	0.468	- 0.1 ± 0.04*
8	+	0.443	- 0.1 ± 0.04*
9	+	0.407	- 0.1 ± 0.03*
10	+	0.362	- 0.1 ± 0.03*
11	+	0.374	-0.08 ± 0.03*
12	+	0.386	-0.09 ± 0.04*
13	+	0.367	-0.04 ± 0.02
14	-	0.323	-0.06 ± 0.03
15	-	0.265	-0.01 ± 0.04
16	-	0.296	-0.04 ± 0.03
17	-	0.296	-0.02 ± 0.01
18	-	0.310	-0.04 ± 0.02

* $P < 0.050$

Table 7.27 Means of the daily total leucocyte counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of total leucocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	10.3 ± 1.6	
1	-	10.8 ± 1.6	-0.5 ± 0.3
2	-	10.1 ± 1.9	0.1 ± 0.4
3	-	8.5 ± 2.6	1.4 ± 0.6
4	+	7.8 ± 3.0	2.4 ± 0.9*
5	+	6.7 ± 2.5	3.3 ± 0.6***
6	+	6.5 ± 2.0	3.5 ± 0.5***
7	+	6.1 ± 1.6	4.0 ± 0.5***
8	+	6.2 ± 1.9	3.5 ± 0.7***
9	+	5.8 ± 1.2	4.2 ± 0.5***
10	+	5.7 ± 1.5	4.5 ± 0.4***
11	+	6.2 ± 1.9	4.0 ± 0.4***
12	+	6.3 ± 1.8	3.9 ± 0.4***
13	+	6.2 ± 1.8	3.8 ± 0.4***
14	+	6.3 ± 2.0	3.9 ± 0.5***
15	+	6.6 ± 1.6	3.6 ± 0.6***
16	+	7.1 ± 1.6	3.3 ± 0.4***
17	-	7.4 ± 1.8	3.0 ± 0.4***
18	-	9.0 ± 1.7	1.8 ± 0.4**

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.28 Total leucocyte count nadirs in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Total leucocyte count nadir ($x10^9/l$)	Days after inoculation
185	3.6	10
137	3.4	4
152	6.0	8
153	5.3	9
154	4.5	10
981	5.1	12
993	5.7	7
64	5.8	7
91	4.2	8
92	4.0	8
mean	4.7	8.3
standard deviation	0.9	2.1

Table 7.29 Means of the daily lymphocyte counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	7.2 ± 1.2	
1	-	7.4 ± 1.3	-0.2 ± 0.04**
2	-	6.8 ± 1.5	0.4 ± 0.3
3	-	5.5 ± 2.2	1.6 ± 0.5**
4	+	4.1 ± 2.1	3.1 ± 0.6***
5	+	3.6 ± 2.0	3.6 ± 0.5***
6	+	3.4 ± 1.5	3.7 ± 0.4***
7	+	3.2 ± 1.2	4.0 ± 0.4***
8	+	3.3 ± 0.9	4.0 ± 0.4***
9	+	3.5 ± 0.8	3.7 ± 0.4***
10	+	3.6 ± 1.0	3.6 ± 0.3***
11	+	4.0 ± 1.2	3.1 ± 0.3***
12	+	4.1 ± 1.5	3.1 ± 0.4***
13	+	4.3 ± 2.0	3.4 ± 0.5***
14	+	4.5 ± 1.4	2.6 ± 0.2***
15	+	5.0 ± 1.1	2.2 ± 0.4***
16	+	5.1 ± 0.9	2.0 ± 0.3***
17	-	5.3 ± 1.0	1.9 ± 0.3***
18	-	6.3 ± 1.6	0.9 ± 0.2**

** $P < 0.010$

*** $P < 0.001$

Table 7.30 Magnitudes of the post-inoculation lymphocytosis in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Magnitude of the lymphocytosis (mm^2)
135	10.5
137	0.5
152	148.5
153	23.0
154	2.0
981	105.5
993	1.5
64	10.0
91	8.0
92	14.0
mean	32.3
standard deviation	51.3

Table 7.31 Lymphocyte count nadirs and magnitudes of the lymphocytopaenia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Magnitude of the lymphocytopaenia (mm^2)
135	1.8	5	3,575
137	2.0	8	4,722
152	1.7	5	2,682
153	1.7	5	4,529
154	3.0	8	3,130
981	2.7	9	2,169
993	3.3	13	2,224
64	2.9	8	3,511
91	1.7	7	2,990
92	2.7	7	2,407
mean	2.3	7.5	3,194
standard deviation	0.6	2.4	899.4

Table 7.32 Means of the daily neutrophil counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of neutrophil counts ($\times 10^9/l$)	Mean and standard error of difference from pre-inoculation count ($\times 10^9/l$)
0	-	2.4 ± 0.6	
1	-	2.9 ± 0.7	-0.4 ± 0.1**
2	-	2.5 ± 1.0	-0.1 ± 0.3
3	-	2.7 ± 1.5	-0.3 ± 0.4
4	+	3.1 ± 1.7	-0.7 ± 0.5
5	+	2.6 ± 1.0	-0.2 ± 0.2
6	+	2.5 ± 0.9	-0.1 ± 0.2
7	+	2.3 ± 0.8	0.05 ± 0.2
8	+	2.3 ± 0.8	0.06 ± 0.3
9	+	1.7 ± 0.5	0.1 ± 0.2
10	+	1.5 ± 0.6	0.8 ± 0.2*
11	+	1.5 ± 0.7	0.7 ± 0.2*
12	+	1.5 ± 0.6	0.8 ± 0.2*
13	+	1.5 ± 0.6	0.8 ± 0.2**
14	+	1.3 ± 0.7	0.9 ± 0.2**
15	+	1.3 ± 0.7	1.0 ± 0.2**
16	+	1.5 ± 0.7	0.9 ± 0.2**
17	-	1.6 ± 0.8	0.7 ± 0.2*
18	-	2.1 ± 0.9	0.3 ± 0.2

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.33 Maximal neutrophil counts and magnitudes of the neutrophilia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Maximal neutrophil count ($x10^9/l$)	Days after inoculation	Magnitude of the neutrophilia (mm^2)
135	4.0	3	470.5
137	2.3	1	109.5
152	3.2	6	561.5
153	3.3	9	528.0
154	2.5	2	211.0
981	3.9	7	445.5
993	7.1	4	1,526.5
64	4.5	2	375.0
91	4.0	4	569.0
92	2.3	4	263.0
mean	3.7	4.2	506.0
standard deviation	1.4	2.4	390.7

Table 7.34 Neutrophil count nadirs and magnitudes of the neutropaenia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia	Magnitude of the neutropaenia (mm^2)
135	0.6	9	2,346.4
137	0.8	9	2,448.0
152	0.6	11	1,289.5
153	0.4	11	905.5
154	0.7	8	3,010.5
981	0.8	2	1,979.0
993	2.1	5	576.0
64	1.2	8	3,310.7
91	0.4	13	3,172.2
92	0.5	12	2,316.8
mean	0.8	8.8	2,135.4
standard deviation	0.5	3.3	947.6

Table 7.35 Medians of the daily eosinophil counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the eosinophil counts ($x10^9/l$)	Mean and standard deviation of difference from the pre-inoculation count ($x10^9/l$)
0	-	0.236	
1	-	0.244	0.02 ± 0.02
2	-	0.309	0.08 ± 0.08
3	-	0.222	0.1 ± 0.08
4	+	0.023	0.3 ± 0.1*
5	+	0.026	0.3 ± 0.1*
6	+	0.076	0.3 ± 0.09*
7	+	0.057	0.3 ± 0.1*
8	+	0.0	0.3 ± 0.1*
9	+	0.0	0.3 ± 0.1*
10	+	0.025	0.3 ± 0.09*
11	+	0.079	0.3 ± 0.1*
12	+	0.071	0.2 ± 0.1*
13	+	0.201	0.2 ± 0.1
14	+	0.130	0.1 ± 0.1
15	+	0.138	0.2 ± 0.1
16	+	0.168	0.2 ± 0.1
17	-	0.240	0.1 ± 0.08
18	-	0.280	0.08 ± 0.1

* $P < 0.050$

Table 7.36 Magnitudes of the eosinopaenia and monocytosis in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
135	5,323	3,023
137	5,276	367
152	4,230	1,012
153	4,574	819
154	4,334	3,070
981	4,090	2,206
993	3,095	744
64	5,505	855
91	2,272	1,800
92	2,648	1,304
Mean	4,134.6	1,520
Standard deviation	1,135.0	965

Table 7.37 Medians of the daily monocyte counts of ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the monocyte counts ($x10^9/l$)	Mean and standard error of difference from the pre-inoculation count ($x10^9/l$)
0	-	0.261	
1	-	0.245	0.03 ± 0.04
2	-	0.259	0.01 ± 0.05
3	-	0.243	0.04 ± 0.03
4	+	0.280	0.02 ± 0.02
5	+	0.283	-0.01 ± 0.02
6	+	0.304	-0.09 ± 0.08
7	+	0.323	-0.04 ± 0.01*
8	+	0.318	-0.03 ± 0.01*
9	+	0.351	-0.08 ± 0.02**
10	+	0.371	-0.06 ± 0.02*
11	+	0.382	-0.07 ± 0.01***
12	+	0.374	-0.08 ± 0.02**
13	+	0.359	-0.06 ± 0.02*
14	+	0.316	-0.02 ± 0.01
15	+	0.300	-0.04 ± 0.02
16	+	0.320	-0.03 ± 0.02
17	-	0.325	-0.02 ± 0.01
18	-	0.320	-0.04 ± 0.02

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.38 Means of the daily total leucocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of total leucocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	9.5 ± 2.7	
1	-	9.6 ± 2.8	-0.2 ± 0.1
2	-	9.2 ± 2.5	0.3 ± 0.3
3	+	8.7 ± 2.1	0.8 ± 0.5
4	+	7.2 ± 2.1	2.2 ± 1.0*
5	+	6.1 ± 2.2	3.0 ± 0.8**
6	+	6.2 ± 1.8	2.9 ± 0.5***
7	+	6.2 ± 1.8	3.0 ± 0.6**
8	+	5.5 ± 1.5	3.6 ± 0.8**
9	+	5.5 ± 1.7	3.4 ± 0.8**
10	+	5.3 ± 1.2	3.9 ± 0.7***
11	+	5.4 ± 1.2	3.8 ± 0.8**
12	+	6.0 ± 1.2	3.3 ± 0.8**
13	+	6.0 ± 1.7[1]	3.3 ± 1.0**
14	+	6.0 ± 1.5[1]	2.9 ± 1.0*
15	+	6.7 ± 1.4[1]	2.5 ± 0.9*
16	+	7.6 ± 1.2[1]	1.2 ± 0.7
17	+	8.0 ± 1.3[1]	1.3 ± 0.7
18	-	9.3 ± 2.7[1]	0.6 ± 0.4

[1]Derived from observations on nine animals

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.39 Total leucocyte count nadirs in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Total lymphocyte count nadir (x 10^9/l)	Days after inoculation
139	3.4	13
140	3.5	8
163	6.1	8
164	4.4	4
95	5.6	12
96	6.2	11
109	7.4	8
113	4.9	9
115	5.5	10
116	4.2	5
mean	5.1	8.8
standard deviation	1.2	2.8

Table 7.40 Means of the daily lymphocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia and significance of changes from the pre-inoculation counts

Day after inoculation	Parasitaemia	Mean and standard deviation of lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	6.6 ± 1.9	
1	-	7.4 ± 1.9	-0.6 ± 0.2*
2	-	6.2 ± 1.7	0.4 ± 0.3
3	+	5.0 ± 1.7	1.6 ± 0.4**
4	+	3.6 ± 0.7	3.0 ± 0.5***
5	+	3.2 ± 1.0	3.4 ± 0.4***
6	+	2.8 ± 2.0	3.8 ± 0.5***
7	+	3.0 ± 0.7	3.6 ± 0.5***
8	+	2.9 ± 0.7	3.7 ± 0.5***
9	+	3.4 ± 1.1	3.1 ± 0.7**
10	+	3.6 ± 0.9	3.0 ± 0.5***
11	+	3.8 ± 1.1	2.8 ± 0.6**
12	+	4.2 ± 1.1	2.3 ± 0.6**
13	+	4.2 ± 1.0[1]	2.3 ± 0.7*
14	+	4.1 ± 1.1[1]	2.5 ± 0.7**
15	+	4.7 ± 1.0[1]	1.7 ± 0.6*
16	+	5.6 ± 1.6[1]	0.9 ± 0.5
17	+	5.8 ± 1.5[1]	0.6 ± 0.5
18	-	6.5 ± 1.7[1]	0.04 ± 0.5

[1] Derived from observations on nine animals

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.41 Magnitudes of the post-inoculation lymphocytosis in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Magnitude of the lymphocytosis (mm^2)
139	3.5
140	51.0
163	3.5
164	80.0
95	108.0
96	66.5
109	69.5
113	54.0
115	50.0
116	8.0
mean	49.4
standard deviation	34.9

Table 7.42 Lymphocyte count nadirs and magnitudes of the lymphocytopaenia in ten lambs inoculated with TBF-infected blood 10^{-1} and PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Magnitude of the lymphocytopaenia (mm^2)
139	1.7	8	4,710
140	1.9	8	4,160
163	2.9	6	3,180
164	2.1	3	1,841
95	3.0	9	2,352
96	2.8	7	2,567
109	3.5	10	2,402
113	1.7	5	1,793
115	3.1	9	4,442
116	1.9	7	2,154
mean	2.5	7.2	2,960
standard deviation	0.6	2.1	1,097.8

Table 7.43 Means of the daily neutrophil counts of ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of neutrophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	2.3 ± 0.9	
1	-	2.0 ± 0.6	0.3 ± 0.2
2	-	2.2 ± 0.8	0.08 ± 0.1
3	+	2.7 ± 0.8	-0.4 ± 0.1*
4	+	2.9 ± 1.2	-0.6 ± 0.3
5	+	2.4 ± 1.3	-0.1 ± 0.2
6	+	2.6 ± 1.0	-0.3 ± 0.2
7	+	2.4 ± 0.9	-0.1 ± 0.1
8	+	2.0 ± 0.5	0.3 ± 0.2
9	+	1.6 ± 0.7	0.6 ± 0.1**
10	+	1.2 ± 0.5	1.1 ± 0.2**
11	+	1.1 ± 0.3	1.2 ± 0.3**
12	+	1.0 ± 0.3	1.2 ± 0.2**
13	+	1.2 ± 0.5	1.0 ± 0.3*
14	+	1.2 ± 0.4	0.9 ± 0.3*
15	+	1.1 ± 0.5	1.1 ± 0.2**
16	+	1.4 ± 1.0	0.7 ± 0.2**
17	+	1.6 ± 1.1	0.6 ± 0.2*
18	-	2.1 ± 1.1	0.3 ± 1.8

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.44 Maximal neutrophil counts and magnitudes of the neutrophilia in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Maximal neutrophil count ($x10^9/l$)	Days after inoculation	Magnitude of the neutrophilia (mm^2)
139	2.5	1	20
140	4.4	4	609
163	2.9	6	954
164	1.9	6	837
95	4.9	5	274
96	3.9	1	277
109	3.9	3	768
113	2.5	4	1,295
115	3.4	1	232
116	2.1	3	240
mean	3.2	3.4	550.6
standard deviation	1.0	1.9	405.5

Table 7.45 Neutrophil count nadirs and magnitudes of the neutropaenia in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia	Magnitude of the neutropaenia (mm^2)
139	0.5	9	4,334
140	0.4	11	2,125
163	0.7	9	2,417
164	0.5	7	1,470
95	1.0	9	2,415
96	1.2	8	1,105
109	1.3	10	983
113	0.9	10	1,015
115	0.9	7	2,388
116	0.9	7	1,571
mean	0.8	8.7	1,982.3
standard deviation	0.2	1.4	1,012.5

Table 7.46 Medians of the daily eosinophil counts of ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the eosinophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.210	
1	-	0.256	0.06 ± 0.06
2	-	0.239	0.05 ± 0.07
3	+	0.176	0.1 ± 0.05
4	+	0.072	0.1 ± 0.05*
5	+	0.0	0.1 ± 0.05*
6	+	0.0	0.1 ± 0.05*
7	+	0.0	0.2 ± 0.05**
8	+	0.053	0.2 ± 0.07**
9	+	0.064	0.2 ± 0.07*
10	+	0.064	0.2 ± 0.07*
11	+	0.145	0.1 ± 0.07
12	+	0.237	-0.03 ± 0.04
13	+	0.295[1]	-0.02 ± 0.06
14	+	0.276[1]	-0.01 ± 0.08
15	+	0.287[1]	0.08 ± 0.07
16	+	0.261[1]	0.01 ± 0.05
17	+	0.252[1]	-0.007 ± 0.05
18	-	0.230[1]	0.02 ± 0.06

[1] Derived from observations on nine animals

* $P < 0.050$

** $P < 0.010$

Table 7.47 Magnitudes of the eosinopaenia and monocytosis in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
139	3,003	2,331
140	2,814	341
163	4,044	2,722
164	3,469	4,524
95	3,248	5,418
96	3,213	3,191
109	2,574	869
113	3,238	1,497
115	5,464	4,450
116	2,269	3,362
Mean	3,334	2,870.4
Standard deviation	893	1,655.2

Table 7.48 Medians of the daily monocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the monocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.161	
1	-	0.175	-0.01 ± 0.02
2	-	0.179	-0.01 ± 0.02
3	+	0.204	-0.01 ± 0.01
4	+	0.207	-0.003 ± 0.02
5	+	0.236	-0.01 ± 0.03
6	+	0.273	-0.04 ± 0.02
7	+	0.335	-0.08 ± 0.03*
8	+	0.327	-0.09 ± 0.03*
9	+	0.341	-0.1 ± 0.02***
10	+	0.327	-0.1 ± 0.02**
11	+	0.305	-0.08 ± 0.01**
12	+	0.352	-0.1 ± 0.02**
13	+	0.341[1]	-0.1 ± 0.02**
14	+	0.344[1]	-0.1 ± 0.02**
15	+	0.359[1]	-0.1 ± 0.02**
16	+	0.342[1]	-0.1 ± 0.02**
17	+	0.358[1]	-0.1 ± 0.02**
18	-	0.337[1]	-0.09 ± 0.02**

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

[1]Derived from observations on nine animals

Table 7.49 Means of the daily total leucocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of total leucocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	10.5 ± 4.8	
1	-	11.5 ± 4.0	-1.1 ± 0.6
2	-	10.6 ± 4.4	-0.1 ± 0.4
3	+	11.4 ± 5.0	-0.7 ± 0.9
4	+	7.7 ± 3.9	2.6 ± 1.4
5	+	7.5 ± 2.8	2.9 ± 1.4
6	+	6.8 ± 2.4	3.5 ± 1.5
7	+	6.2 ± 2.6	4.3 ± 1.5*
8	+	6.3 ± 1.8	4.2 ± 1.1**
9	+	5.3 ± 1.4	5.3 ± 1.4**
10	+	5.6 ± 2.7	4.1 ± 1.4*
11	+	5.9 ± 1.9	4.5 ± 1.4*
12	+	6.8 ± 2.5	3.6 ± 1.4*
13	+	6.8 ± 2.0	3.7 ± 1.5*
14	+	7.3 ± 2.4	3.1 ± 1.4
15	+	7.7 ± 2.2	2.7 ± 1.4
16	+	8.0 ± 2.2	2.4 ± 1.4
17	+	8.6 ± 2.3	1.8 ± 1.4
18	-	9.5 ± 1.9	1.0 ± 1.4

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.50 Total leucocyte count nadirs in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Total leucocyte count nadir ($x10^9/l$)	Days after inoculation
68	5.3	5
984	5.2	8
990	2.0	10
78	5.1	13
79	6.1	9
992	5.8	8
797	7.5	9
62	4.7	7
101	2.4	4
103	2.7	7
mean	4.7	8.0
standard deviation	1.7	2.5

Table 7.51 Means of the daily lymphocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of lymphocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	6.5 ± 2.3	
1	-	7.9 ± 2.2	-1.3 ± 0.5*
2	-	6.7 ± 2.7	-0.2 ± 0.6
3	+	6.5 ± 3.8	0.01 ± 0.8
4	+	4.4 ± 2.3	2.06 ± 0.6*
5	+	4.1 ± 1.9	2.4 ± 0.7**
6	+	3.5 ± 1.6	3.0 ± 0.8**
7	+	3.0 ± 1.1	3.4 ± 0.7***
8	+	3.8 ± 1.1	2.7 ± 0.5***
9	+	3.3 ± 1.0	3.2 ± 0.6***
10	+	4.3 ± 1.8	1.7 ± 0.7*
11	+	4.7 ± 2.1	1.7 ± 0.7*
12	+	5.2 ± 2.2	1.2 ± 0.8
13	+	5.4 ± 1.9	1.0 ± 0.9
14	+	5.6 ± 1.8	1.0 ± 0.5
15	+	5.5 ± 1.7	0.9 ± 0.7
16	+	5.8 ± 1.7	0.6 ± 0.5
17	+	6.1 ± 1.7	0.4 ± 0.6
18	-	6.4 ± 2.0	0.1 ± 0.8

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.52 Magnitudes of the post-inoculation lymphocytosis in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Magnitude of the lymphocytosis (mm^2)
68	105.5
984	143.0
990	234.0
78	52.5
79	232.5
992	168.0
797	119.0
62	43.5
101	75.0
103	80.0
mean	125.3
standard deviation	68.6

Table 7.53 Lymphocyte count nadirs and magnitudes of the lymphocytopaenia in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Magnitude of lymphocytopaenia (mm^2)
68	2.6	7	2,520
984	2.5	9	1,972
990	1.6	9	530
78	2.8	4	1,761
79	2.8	6	2,103
992	3.1	13	1,263
797	3.8	7	2,303
62	2.0	11	5,210
101	1.4	4	2,495
103	1.1	7	3,096
mean	2.4	7.7	2,325.3
standard deviation	0.8	2.8	1,239.4

Table 7.54 Means of the daily neutrophil counts of ten lambs inoculated with TBF-infected blood 10^{-1} and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Mean and standard deviation of neutrophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	3.3 ± 2.8	
1	-	3.8 ± 3.5	-0.5 ± 0.4
2	-	3.2 ± 3.4	0.1 ± 0.4
3	+	4.6 ± 3.0	-1.3 ± 0.4*
4	+	2.7 ± 1.7	0.6 ± 0.9
5	+	2.7 ± 1.4	0.6 ± 0.9
6	+	2.4 ± 1.0	0.8 ± 0.8
7	+	2.3 ± 1.3	0.9 ± 1.0
8	+	1.8 ± 0.7	1.4 ± 0.7
9	+	1.3 ± 0.5	2.0 ± 0.5*
10	+	0.9 ± 0.3	2.3 ± 0.3*
11	+	0.6 ± 0.5	2.6 ± 0.5**
12	+	0.9 ± 0.5	2.4 ± 0.5*
13	+	0.9 ± 0.6	2.1 ± 0.6*
14	+	1.1 ± 0.7	2.1 ± 0.8*
15	+	1.4 ± 0.5	1.8 ± 0.7*
16	+	1.5 ± 0.5	1.7 ± 0.7*
17	+	1.8 ± 0.6	1.4 ± 0.8
18	-	2.5 ± 0.9	0.8 ± 0.7

* $P < 0.050$

** $P < 0.010$

Table 7.55 Maximal neutrphil counts and magnitudes of the neutrophilia in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Maximal neutrophil count ($x10^9/l$)	Days after inoculation	Magnitude of the neutrophilia (mm^2)
68	6.3	1	493.0
984	3.3	3	1,712.0
990	1.8	4	150.5
78	5.4	4	583.0
79	7.3	1	45.0
992	5.7	3	1,593.5
797	6.4	4	755.5
62	8.8	1	20.0
101	1.5	2	28.5
103	2.2	3	150.5
mean	4.8	2.6	553.1
standard deviation	2.5	1.2	633.5

Table 7.56 Neutrophil count nadirs and magnitudes of the neutropaenia in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia	Magnitude of the neutropaenia (mm^2)
68	0.6	8	4,489.0
984	0.4	10	2,000.0
990	0.4	7	1,228.5
78	0.3	7	3,626.5
79	0.2	8	5,691.5
992	0.5	3	1,037.0
797	0.3	8	2,888.5
62	1.5	11	6,837.5
101	0.5	9	2,784.3
103	0.5	10	2,647.5
mean	0.5	8.1	3,323
standard deviation	0.3	2.2	1,877

Table 7.57 Medians of the daily eosinophil counts of ten lambs inoculated with TBF-infected blood 10^{-1} and significance of changes from the pre-inoculation count

Days after inoculation	Parasitaemia	Medians of the eosinophil counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.157	
1	-	0.264	0.2 ± 0.09
2	-	0.205	0.1 ± 0.06
3	+	0.115	0.01 ± 0.05
4	+	0.047	0.05 ± 0.08
5	+	0.0	0.1 ± 0.06
6	+	0.0	0.1 ± 0.05
7	+	0.0	0.1 ± 0.03**
8	+	0.077	0.1 ± 0.02***
9	+	0.080	0.1 ± 0.03**
10	+	0.033	0.1 ± 0.02***
11	+	0.058	0.1 ± 0.02***
12	+	0.073	0.1 ± 0.03**
13	+	0.070	0.1 ± 0.03*
14	+	0.131	0.01 ± 0.03
15	+	0.202	-0.05 ± 0.03
16	+	0.198	-0.01 ± 0.04
17	+	0.205	-0.02 ± 0.03
18	-	0.245	-0.06 ± 0.04

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 7.58 Magnitudes of the eosinopaenia and monocytosis in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Sheep No.	Eosinopaenia (mm^2)	Monocytosis (mm^2)
68	3,816	12
984	2,442	147
990	3,605	5,806
78	4,126	174
79	6,178	6,360
992	4,203	2,550
797	3,058	41
62	3,061	200
101	3,789	2,729
103	4,074	4,615
Mean	3,835.2	2,263.4
Standard deviation	997.6	2,544.5

Table 7.59 Medians of the daily monocyte counts of ten lambs inoculated with TBF-infected blood 10^{-1} and significance of changes from the pre-inoculation counts

Days after inoculation	Parasitaemia	Medians of the monocyte counts ($x10^9/l$)	Mean and standard error of difference from pre-inoculation count ($x10^9/l$)
0	-	0.247	
1	-	0.297	0.01 ± 0.05
2	-	0.234	0.07 ± 0.09
3	+	0.286	0.1 ± 0.07
4	+	0.326	-0.02 ± 0.1
5	+	0.388	-0.02 ± 0.07
6	+	0.587	-0.01 ± 0.08
7	+	0.432	-0.04 ± 0.07
8	+	0.458	-0.05 ± 0.1
9	+	0.413	-0.03 ± 0.1
10	+	0.365	-0.01 ± 0.1
11	+	0.512	-0.07 ± 0.06
12	+	0.534	-0.06 ± 0.06
13	+	0.485	-0.04 ± 0.07
14	+	0.438	-0.06 ± 0.09
15	+	0.356	0.02 ± 0.09
16	+	0.341	0.05 ± 0.07
17	+	0.322	0.07 ± 0.05
18	-	0.328	0.04 ± 0.04

Table 7.60 Haematological parameters: significant subsets of the experimental groups

Parameter	Variance ratio (d.f. 4, 45)	Subsets significant at the one percent level of probability
Magnitude of the lymphocytosis	4.10**	C B D E A
Magnitude of the lymphocytopaenia	11.25**	A / E D C B
Magnitude of the neutrophilia	5.68**	A C D E B
Magnitude of the neutropaenia	21.53**	A / D C B E
Magnitude of the eosinopaenia	15.51**	A / D E C B
Magnitude of the monocytosis	3.65*	A C B E / D

* $P < 0.050$

** $P < 0.010$

A = Group inoculated with PI-3 virus alone

B = Group inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

C = Group inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

D = Group inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF-parasitaemia

E = Group inoculated with TBF-infected blood 10^{-1} alone

Table 7.61 Parameters of the parasitaemia in ten lambs inoculated with TBF-infected blood 10^{-1}

Sheep No.	Prepatent period (days)	Maximal parasitaemia ($\log_{10}$ infected neutrophils/l)	Days after the onset of visible parasitaemia	Duration of visible parasitaemia (days)	Magnitude of the parasitaemia (mm^2)
68	2	9.40	1	7	15,382
984	4	9.34	1	6	13,355
990	3	8.95	0	8	16,765
78	2	9.36	2	9	20,045
79	2	9.40	2	8	17,682
992	2	9.52	0	9	19,230
797	2	9.66	1	7	15,922
62	7	9.10	1	9	19,460
101	2	8.94	4	9	18,852
103	2	8.81	3	10	20,642
mean	2.8	9.2	1.5	8.2	17,733.7
standard deviation	1.6	0.3	1.2	1.2	2,338

Table 7.62 Parameters of the parasitaemia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Sheep No.	Prepatent period (days)	Maximal parasitaemia (log_{10} infected neutrophils/l)	Days after the onset of visible parasitaemia	Duration of visible parasitaemia (days)	Magnitude of the parasitaemia (mm^2)
133	3	9.35	2	7	15,465
134	3	9.46	1	10	22,182
149	2	9.35	0	7	15,902
150	2	9.48	1	8	17,802
151	2	9.44	0	11	24,342
988	2	9.00	2	8	17,207
994	3	8.80	4	9	19,262
63	3	9.35	0	9	19,572
89	2	9.37	2	10	21,745
90	2	9.29	1	11	23,837
mean	2.4	9.30	1.3	9.0	17,776.7
standard deviation	0.5	0.2	1.2	1.4	7,005.6

Table 7.63 Parameters of the parasitaemia in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Sheep No.	Prepatent period (days)	Maximal parasitaemia (log_{10} infected neutrophils/l)	Days after the onset of visible parasitaemia	Duration of visible parasitaemia	Magnitude of the parasitaemia (mm^2)
135	3	9.21	1	8	17,595
137	3	9.13	1	10	21,555
152	3	9.15	1	8	17,412
153	4	9.12	2	10	20,887
154	4	9.37	1	8	17,787
981	6	9.05	1	6	13,115
993	8	8.81	6	8	17,255
64	3	9.31	2	10	22,177
91	3	9.34	2	9	20,100
92	3	9.27	2	9	19,960
means	4.0	9.1	1.9	8.6	18,784
standard deviation	1.7	0.1	1.5	1.3	2,697

Table 7.64 Parameters of the parasitaemia in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Sheep No.	Prepatent period (days)	Maximal parasitaemia (log_{10} infected neutrophils/l)	Days after the onset of visible parasitaemia	Duration of visible parasitaemia (days)	Magnitude of the parasitaemia (mm^2)
139	3	8.98	0	10	21,612
140	3	9.29	0	9	19,790
163	2	9.36	3	7	15,680
164	2	9.08	3	7	15,317
95	2	9.49	1	15	32,265
96	2	9.31	2	10	22,380
109	2	9.32	1	10	21,792
113	2	9.20	1	10	21,025
115	2	9.33	1	9	19,475
116	2	9.04	1	10	21,027
mean	2.2	9.20	1.3	9.7	20,886
standard deviation	0.4	0.1	1.0	2.2	4,669

Table 7.65 Parameters of the parasitaemia: significant subsets of the experimental groups

Parameter	Variance ratio (d.f. 3, 36)	Subsets significant at the 5% level of probability
Prepatent period	4.37*	\D B E/ \C/
Maximal parasitaemia	0.09	\C D E B/
Day of occurrence of maximal parasitaemia	0.48	\B D E C/
Duration of visible parasitaemia	1.60	\E C B D/
Magnitude of parasitaemia	0.76	\D B C E/

* $P < 0.050$

B = Group inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

C = Group inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

D = Group inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of TBF parasitaemia

E = Group inoculated with TBF-infected blood 10^{-1} alone

Table 7.66 Recovery of parainfluenza-3 virus from nasal swabs

Experimental group	No. of lambs	No. of lambs from which PI-3 virus was recovered												
		Days after virus inoculation												
		0	1	2	3	4	5	6	7	8	9	10	11	12
PI-3 virus alone	10	0	0	0	9	10	10	5	3	0	0	0	0	0
Simultaneous PI-3 virus and 10^{-1} TBF	10	0	0	0	7	10	10	10	9	6	4	3	0	0
Simultaneous PI-3 virus and 10^{-3} TBF	10	0	0	0	7	10	10	10	9	6	3	2	0	0
TBF 10^{-1} and PI-3 virus at the onset of TBF parasit-aemia	10	0	0	3	10	10	10	10	10	9	9	8	4	0

Table 7.67 Duration of virus excretion in days in groups of ten lambs inoculated with parainfluenza-3 virus alone or in combination with TBF

PI-3 virus alone	Simultaneous PI-3 virus and 10^{-1} TBF	Simultaneous PI-3 virus and 10^{-3} TBF	TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia
3	4	5	7
3	4	5	6
5	8	8	10
3	8	8	9
3	8	7	10
3	5	4	10
3	5	4	8
5	7	6	9
5	4	5	8
4	6	5	8
mean 3.7	5.9	5.7	8.5
standard deviation 1.0	1.8	1.5	1.4

Table 7.68 Significant subsets at the one percent level of probability of mean durations of virus excretion in days

Subsets	Mean and standard deviation
PI-3 virus alone	3.7 ± 1.0
PI-3 virus + TBF 10^{-3} and PI-3 virus + TBF 10^{-1}	5.8 ± 1.6
TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia	8.5 ± 1.4

Table 7.69 Mean HI-antibody titres: significant subsets of the experimental groups.

Days after virus inoculation	Variance ratio	significant subsets at the one percent level of probability	
7	$F^{3}_{36} = 8.46^{**}$	\ B C D /	\ A /
14	$F^{3}_{35} = 5.66^{**}$	\ D B C /	\ A /
21	$F^{3}_{34} = 12.36^{**}$	\ D B C /	\ A /

** $p < 0.010$

A = Group inoculated with PI-3 virus alone

B = Group inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

C = Group inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

D = Group inoculated with TBF 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Table 7.70 Virus neutralising antibody titres: significant subsets of the experimental groups

Days after virus inoculation	Variance ratio	significant subsets at the one percent level of probability
7	$F^{3}_{28} = 1.05$	\ D C B A /
14	$F^{3}_{28} = 1.37$	\ D C B A /
21	$F^{3}_{28} = 5.85^{**}$	\ D C B / \ A /

** $p < 0.010$

A = Group inoculated with PI-3 virus alone

B = Group inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

C = Group inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

D = Group inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Table 7.71 Comparison of the clinical and parasitological parameters of tick-borne fever in two groups of sheep

Parameter	Original eight sheep	Ten sheep (Group E)	$t_{(16)}$
Incubation period (days)	2.5 ± 0.5	2.0 ± 0.6	1.893
Maximal temperature (°C)	41.5 ± 0.4	41.7 ± 0.2	1.388
Days of occurrence of maximal temperature	1.7 ± 0.8	1.7 ± 1.4	0.0
Duration of fever (days)	6.2 ± 1.4	6.6 ± 2.5	0.404
Magnitude of fever (mm^2)	1,871.8 ± 586.6	2,108 ± 592	0.847
Prepatent period (days)	3.0 ± 0.0	2.8 ± 1.6	0.351
Maximal parasitaemia ($\log_{10}$ infected neutrophils/l)	9.2 ± 0.1	9.2 ± 0.3	0.0
Day of occurrence of maximal parasitaemia	1.7 ± 1.9	1.5 ± 1.2	0.272
Duration of visible parasitaemia (days)	7.5 ± 0.7	8.2 ± 1.2	1.458
Magnitude of the parasitaemia (mm^2)	16,403 ± 1,528	17,733 ± 2,338	1.385

Table 7.72 Comparison of the haematological parameters of tick-borne fever in two groups of sheep

Parameter	Original eight sheep	Ten sheep (Group E)	$t_{(16)}$
Magnitude of lymphocytosis (mm^2)	138.5 ± 109.7	125.3 ± 68.6	0.312
Magnitude of lymphocytopaenia (mm^2)	2,989 ± 859	2,325 ± 1,239	1.283
Magnitude of neutrophilia (mm^2)	450 ± 385.6	553 ± 633	0.403
Magnitude of neutropaenia (mm^2)	3,598 ± 933	3,323 ± 1,877	0.377
Magnitude of eosinopaenia (mm^2)	4,607 ± 787.4	3,835 ± 997	1.785
Magnitude of monocytosis (mm^2)	1,955 ± 1,717.8	2,263 ± 2,544	0.292

Fig. 7.1 Mean febrile reactions in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

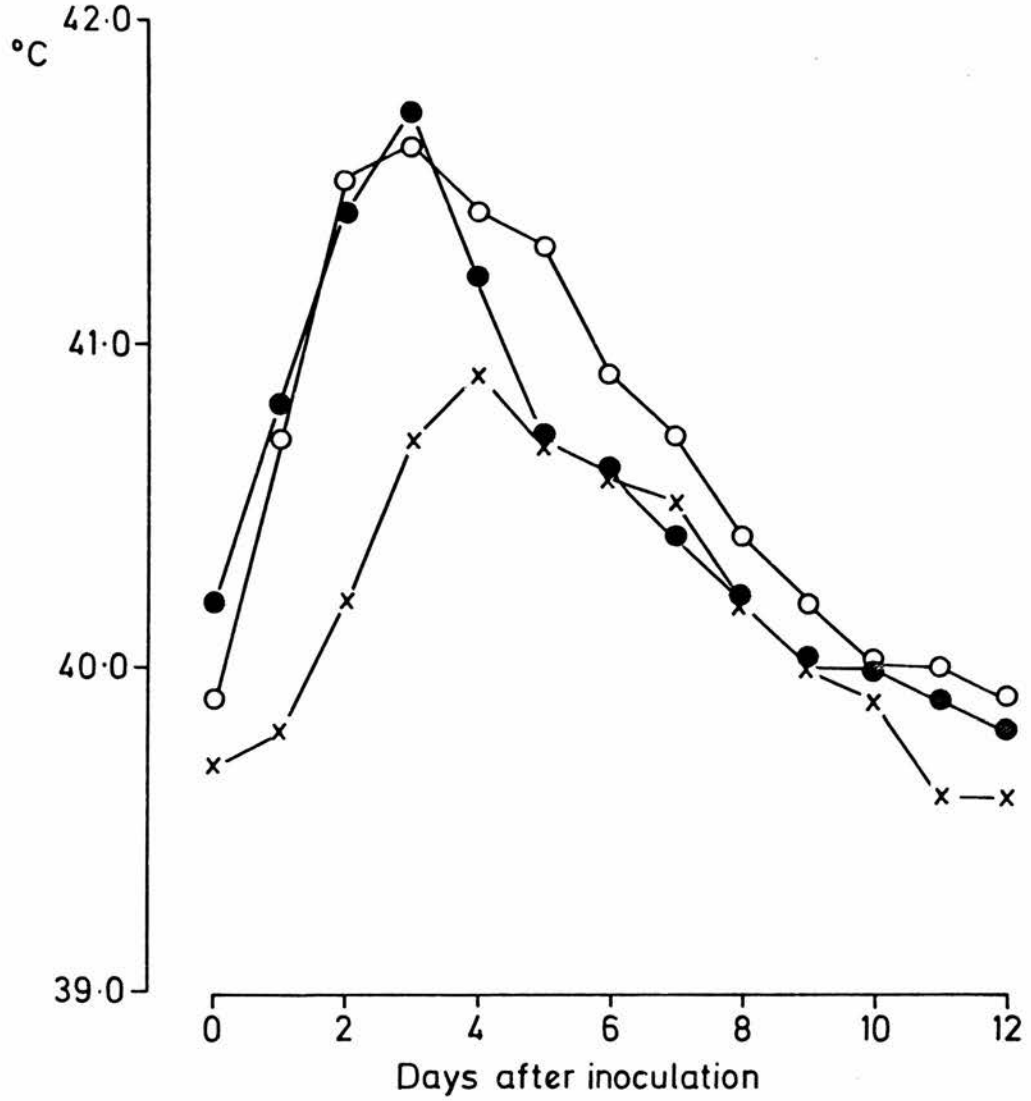

°C
42·0
41·0
40·0
39·0
0
2
4
6
8
10
12
Days after inoculation

Fig. 7.2 Mean febrile reactions in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

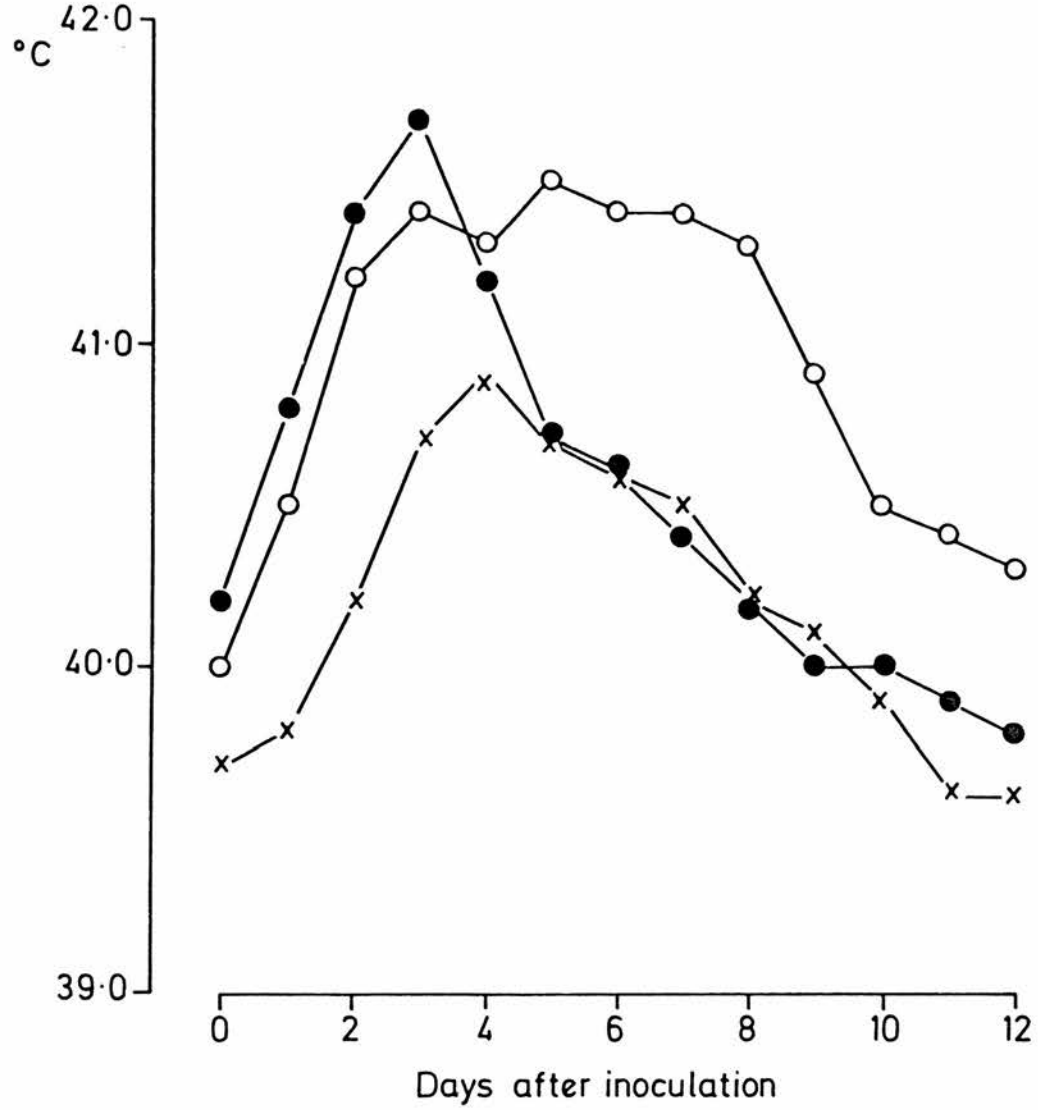

°C
42·0
41·0
40·0
39·0
0
2
4
6
8
10
12
Days after inoculation

Fig. 7.3 Mean febrile reactions in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)

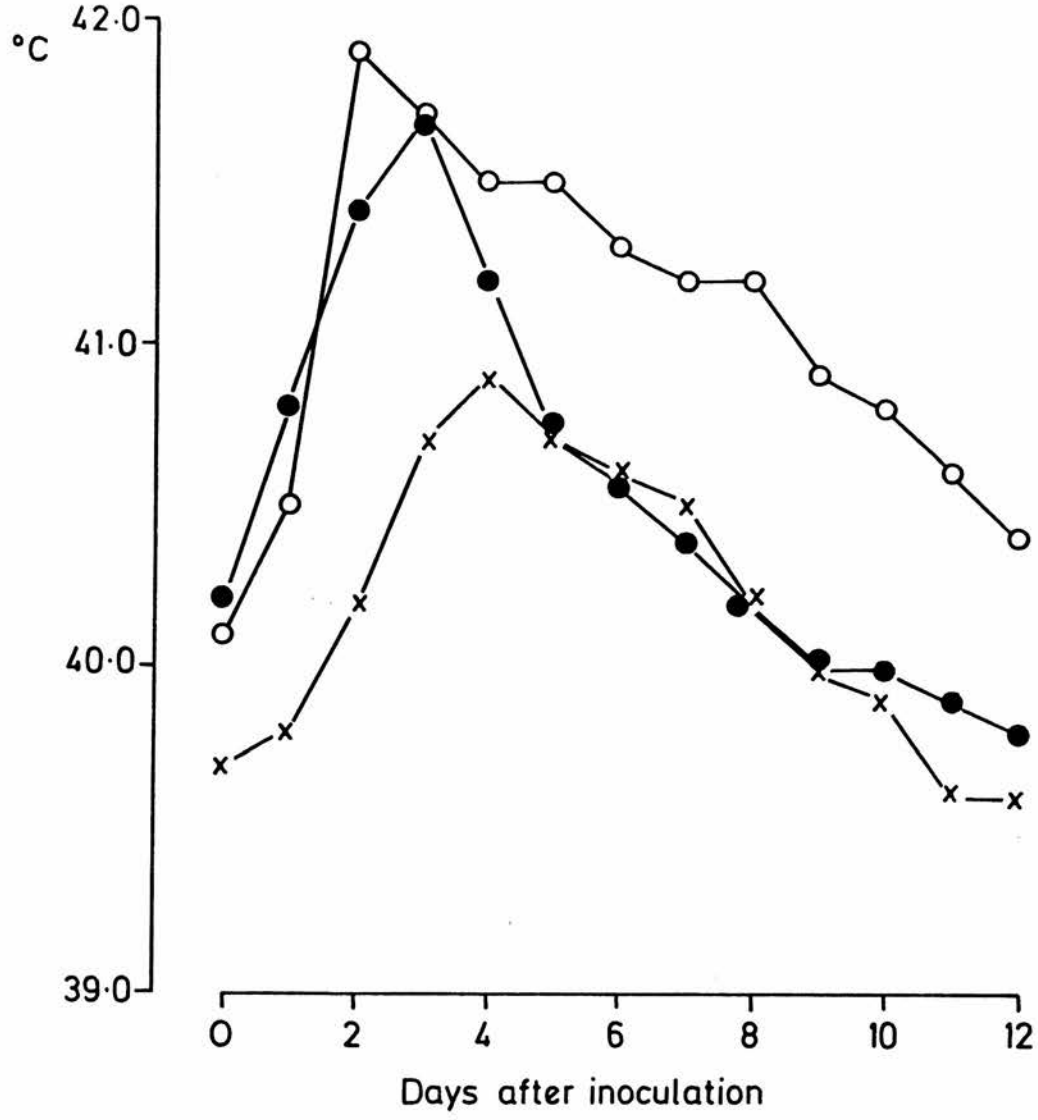

°C
42·0
41·0
40·0
39·0
0
2
4
6
8
10
12
Days after inoculation

Fig. 7.4 Mean daily lymphocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

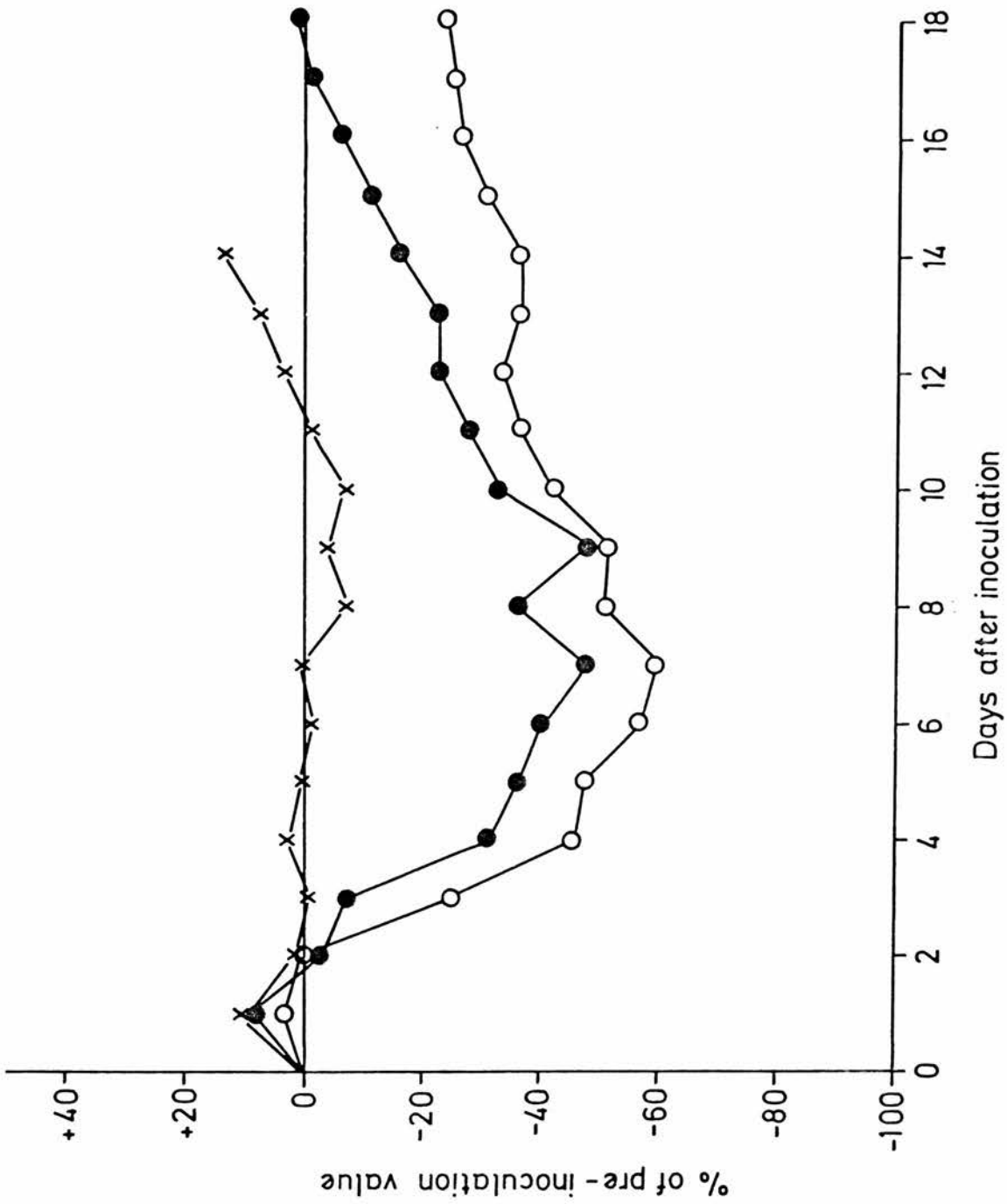

+40
+20
0
-20
-40
-60
-80
-100
% of pre-inoculation value
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.5 Mean daily neutrophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

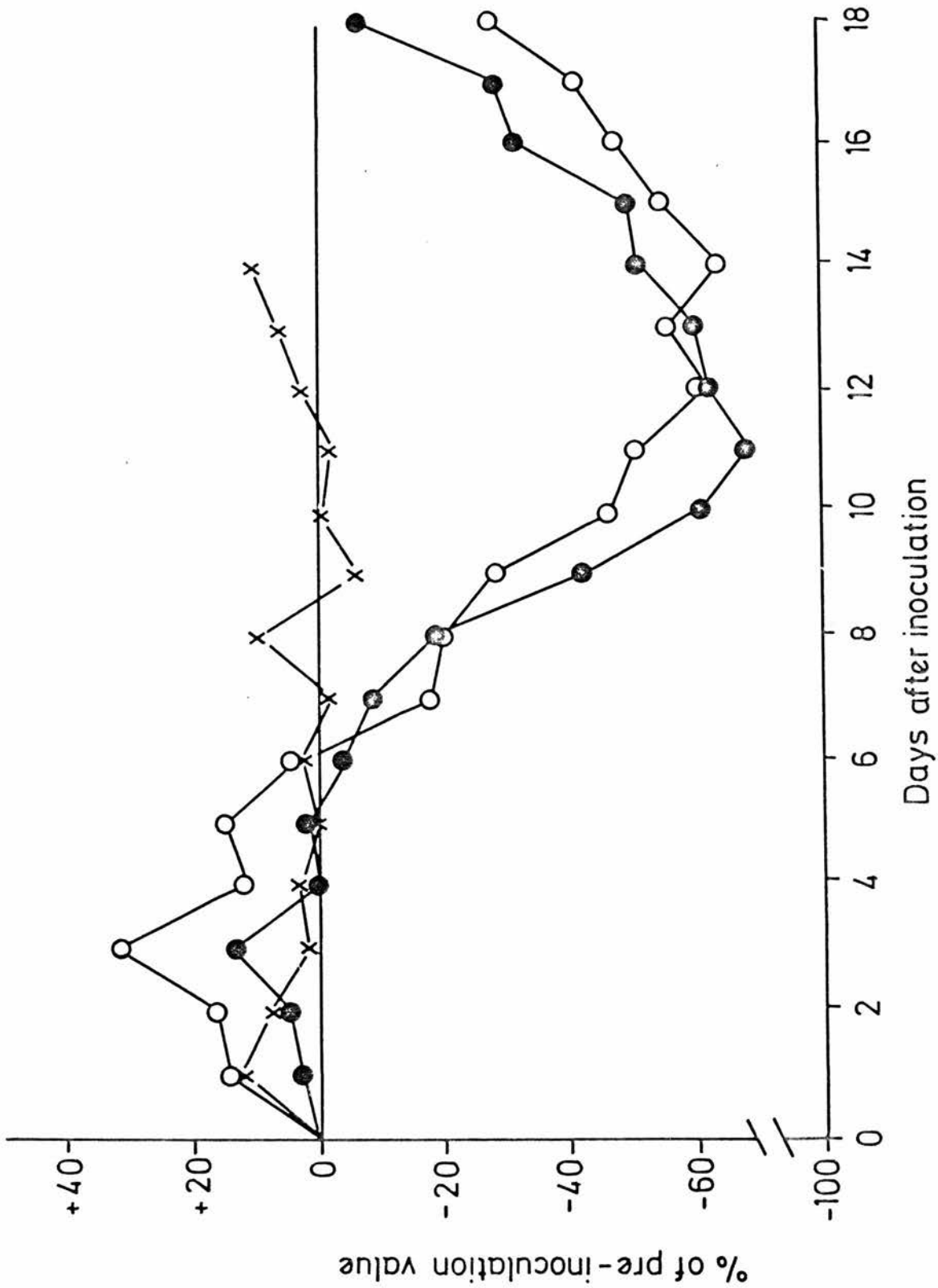

% of pre-inoculation value
+40
+20
0
-20
-40
-60
-100
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.6 Mean daily eosinophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

○ PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

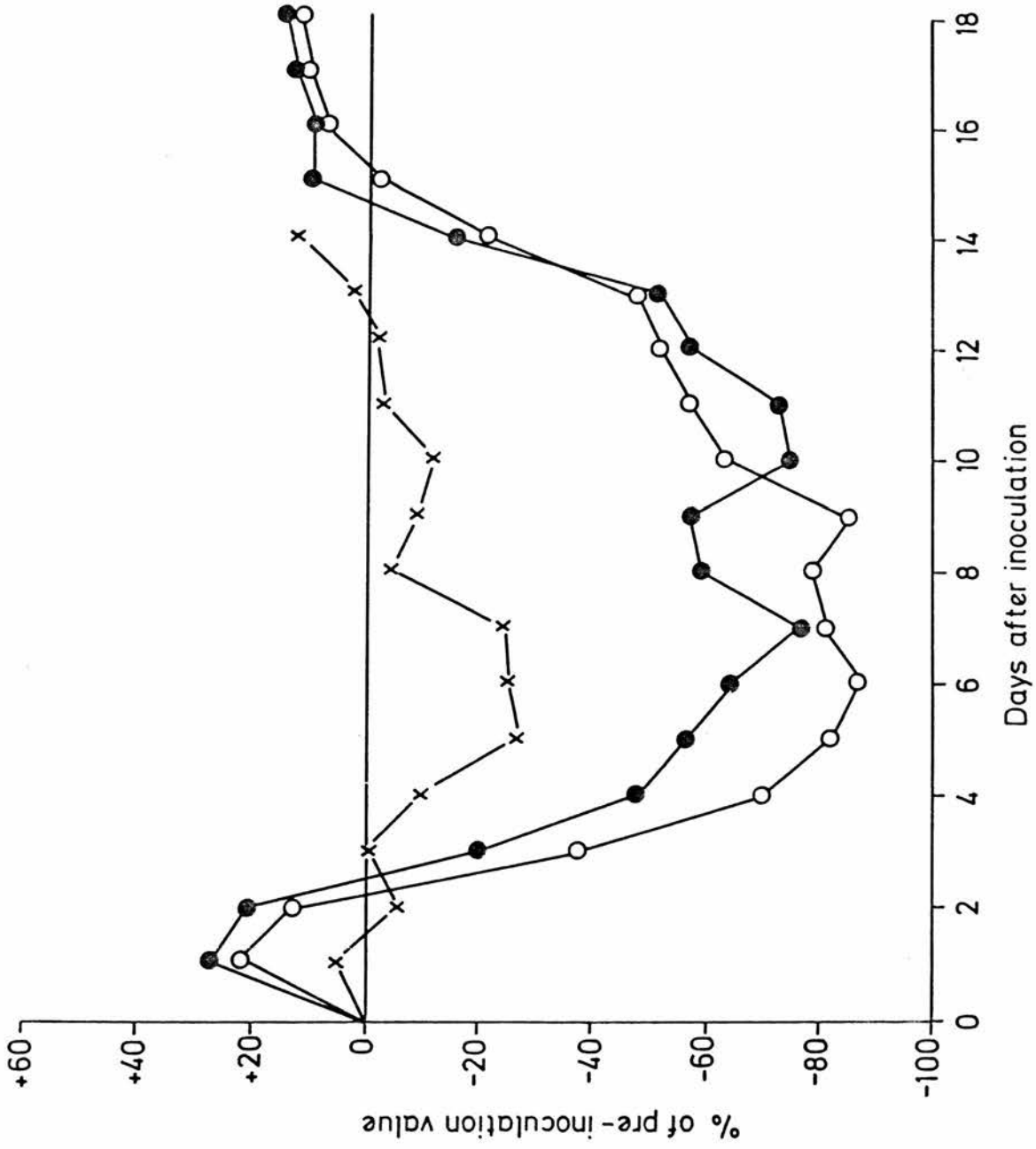

+60
+40
+20
0
-20
-40
-60
-80
-100
% of pre-inoculation value
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.7 Mean daily monocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

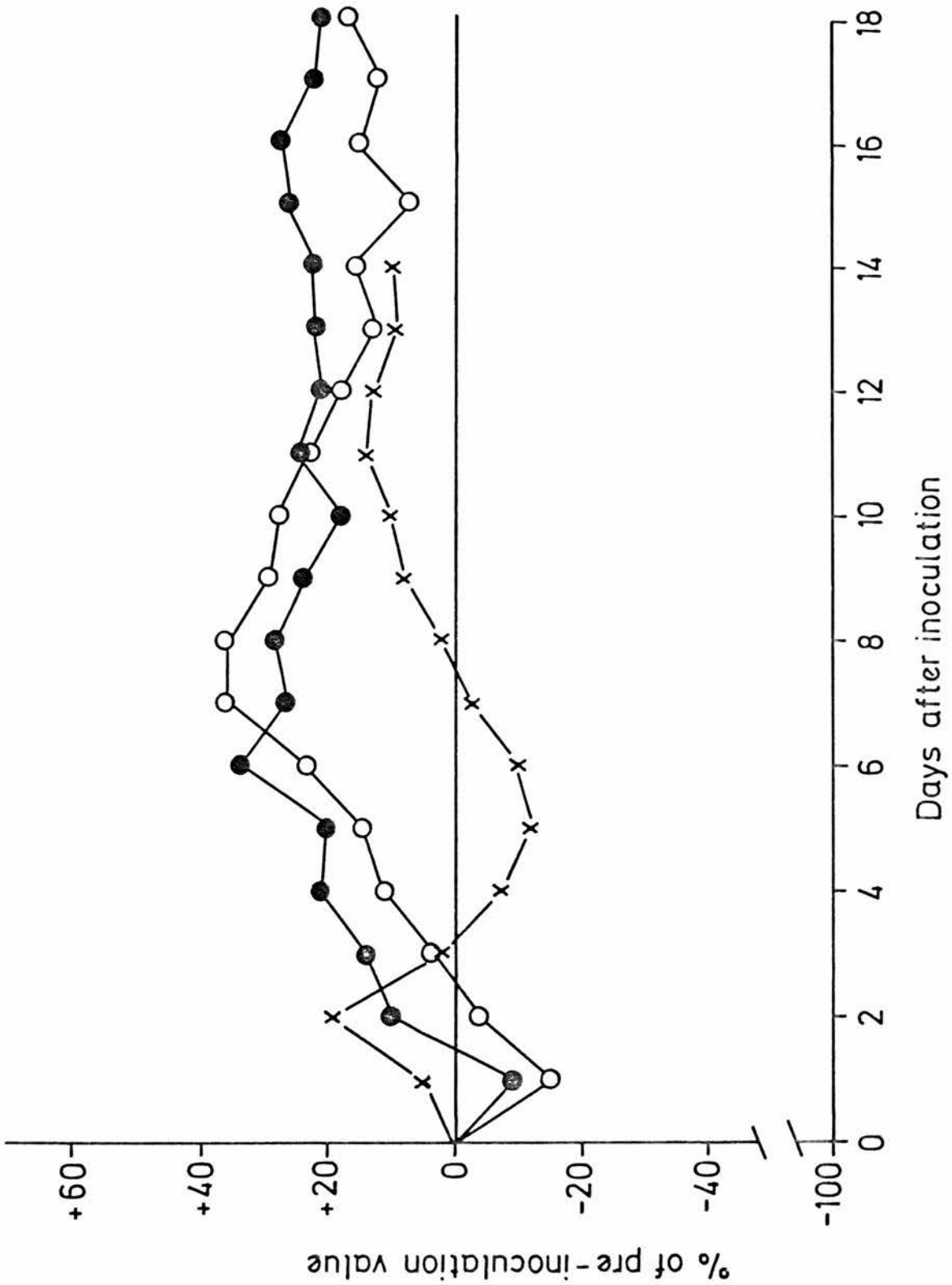

+60
+40
+20
0
-20
-40
-100
0
2
4
6
8
10
12
14
16
18
% of pre-inoculation value
Days after inoculation

Fig. 7.8 Mean daily lymphocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

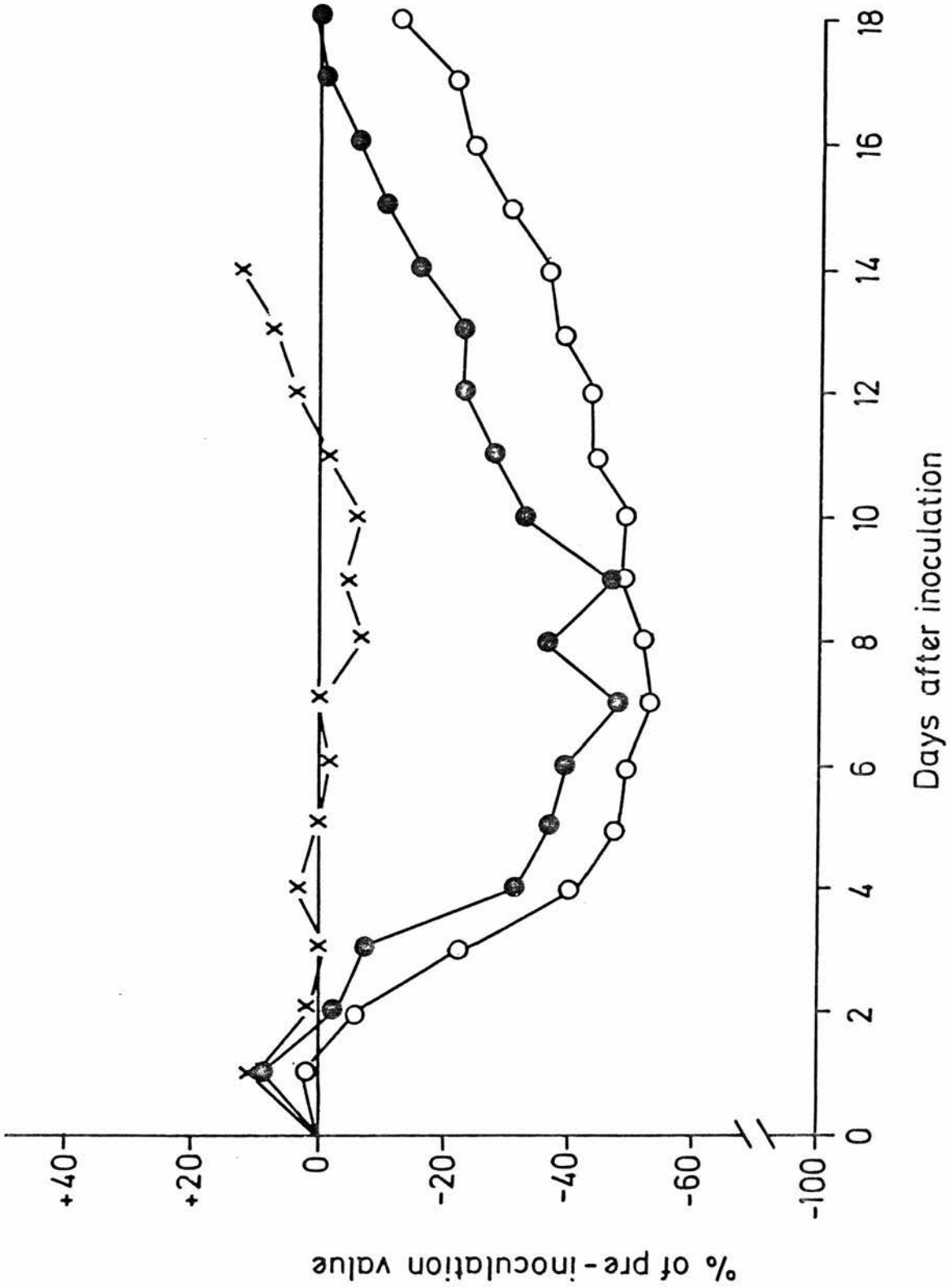

% of pre-inoculation value
+40
+20
0
-20
-40
-60
-100
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.9 Mean daily neutrophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

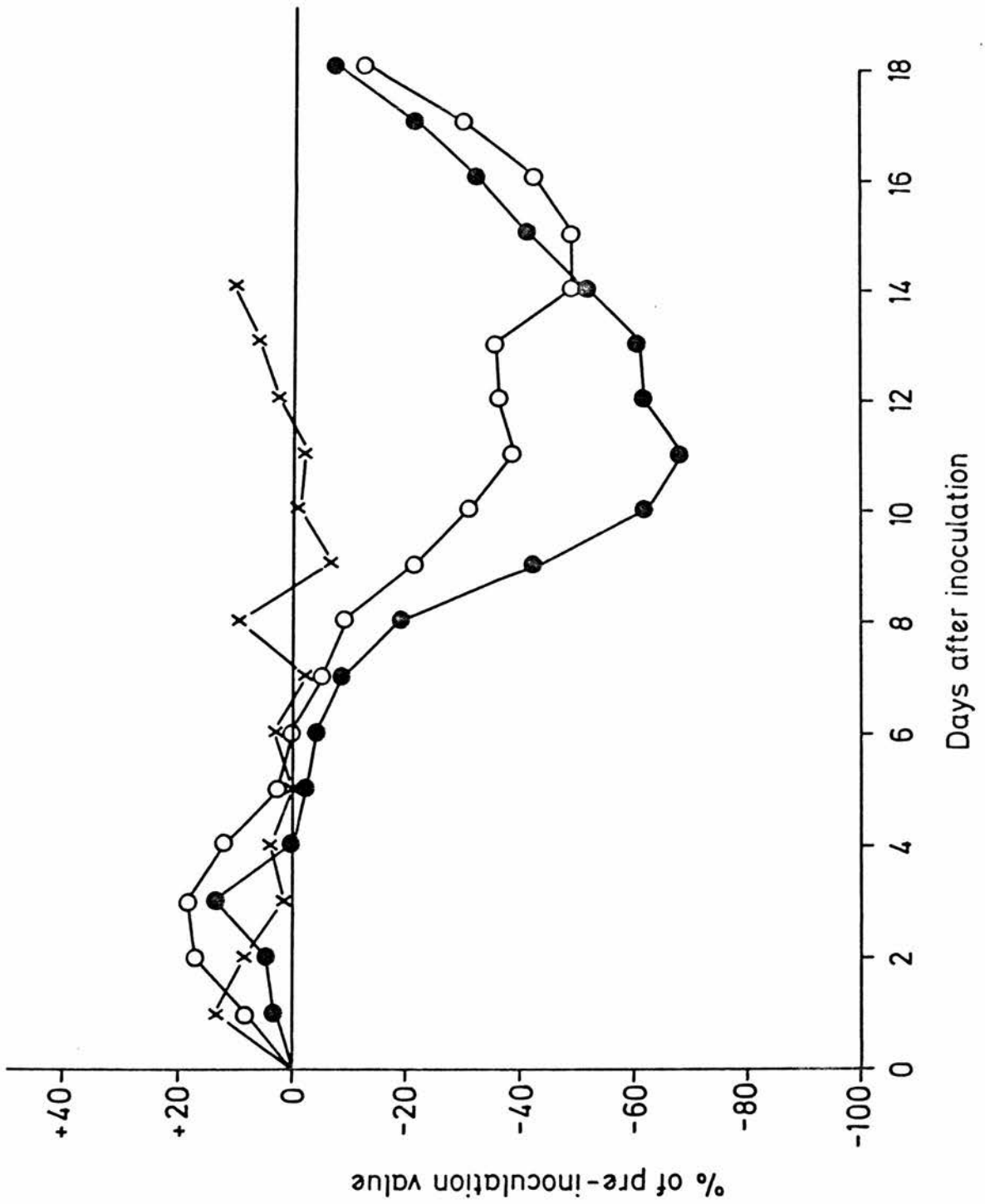

% of pre-inoculation value
+40
+20
0
-20
-40
-60
-80
-100
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.10 Mean daily eosinophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

X PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

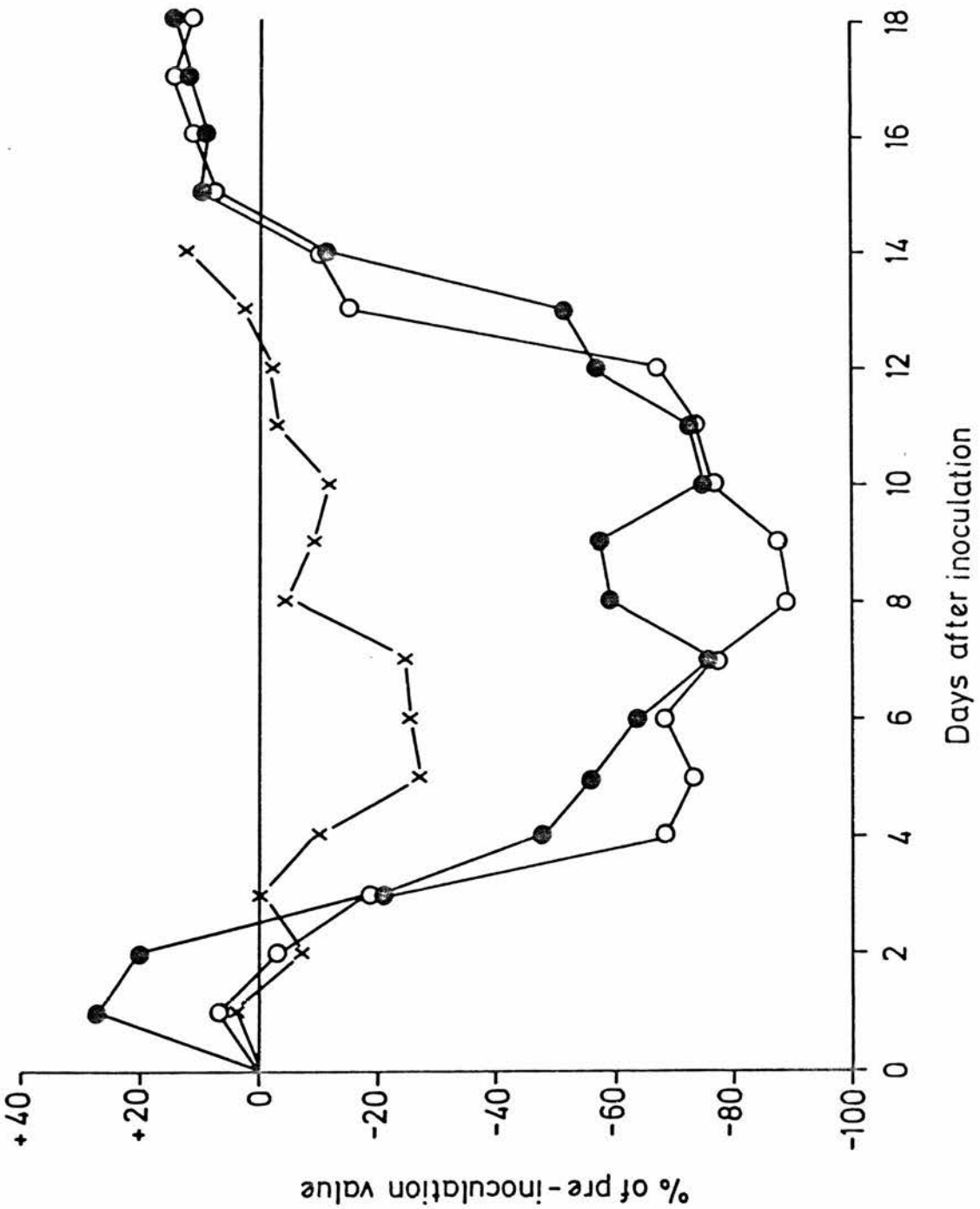

+40
+20
0
-20
-40
-60
-80
-100
% of pre-inoculation value
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.11 Mean daily monocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

x PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

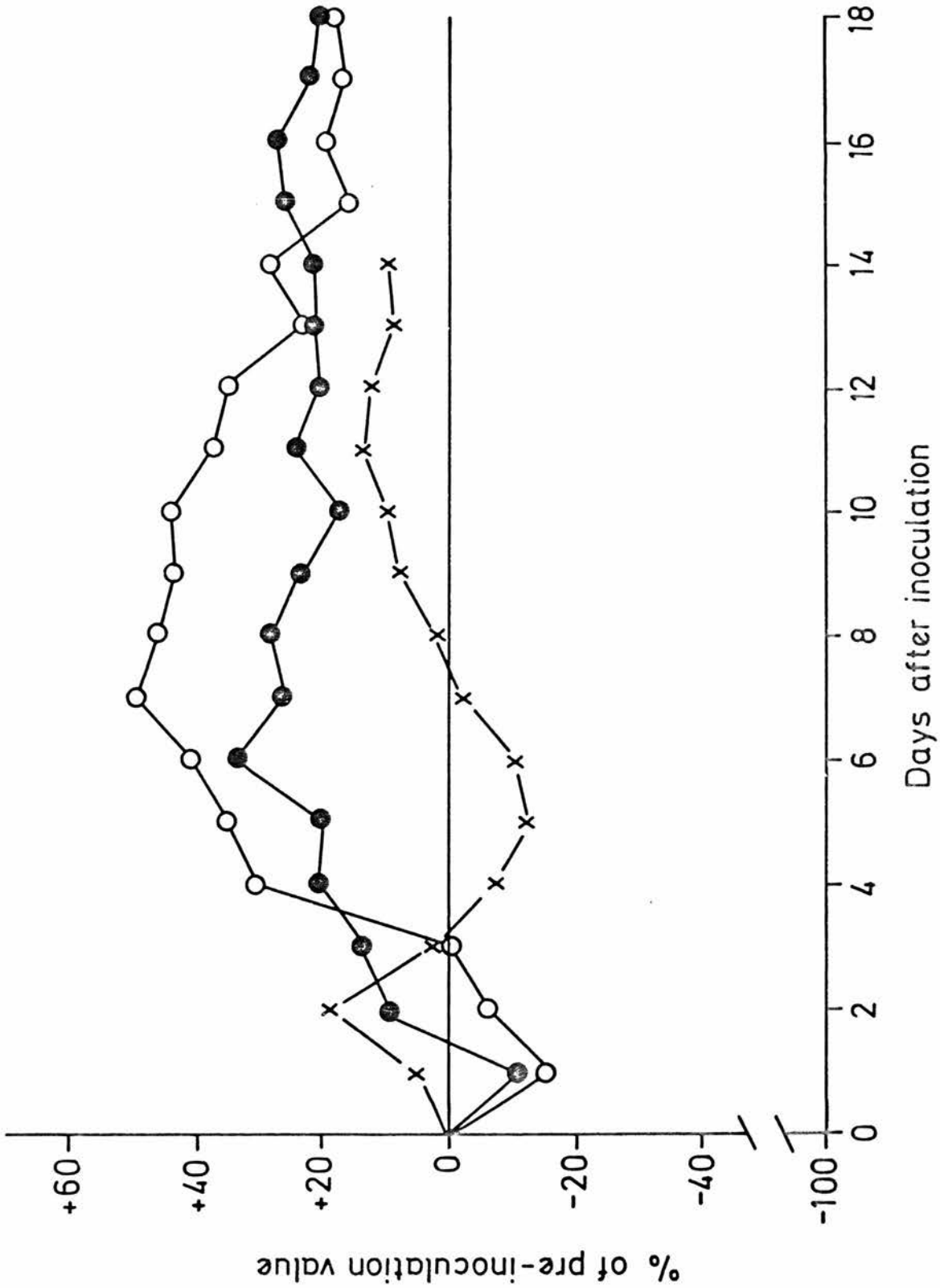

+60
+40
+20
0
-20
-40
-100
0
2
4
6
8
10
12
14
16
18
% of pre-inoculation value
Days after inoculation

Fig. 7.12 Mean daily lymphocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

x PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)

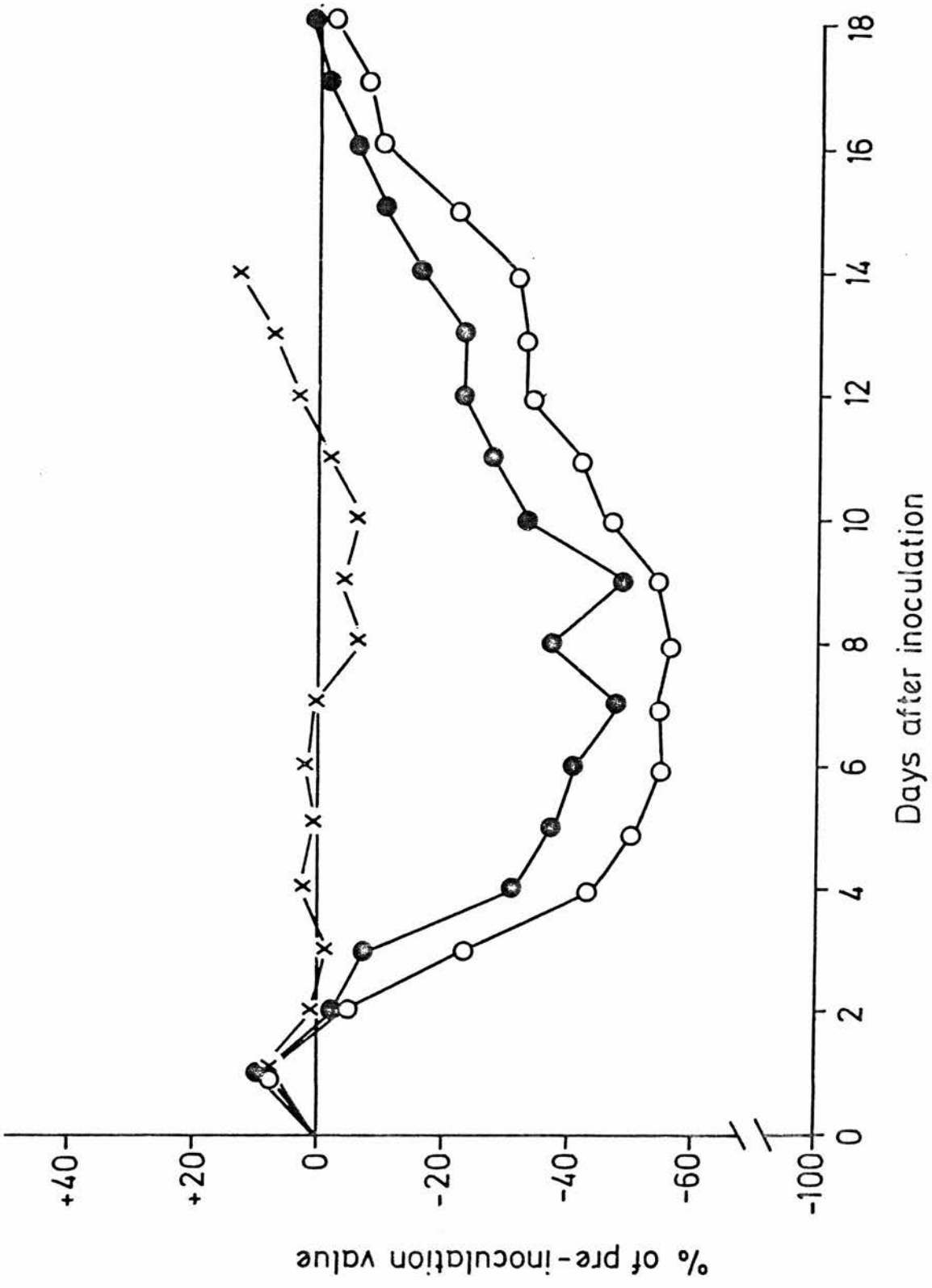

% of pre-inoculation value
+40
+20
0
-20
-40
-60
-100
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.13 Mean daily neutrophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

x PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)

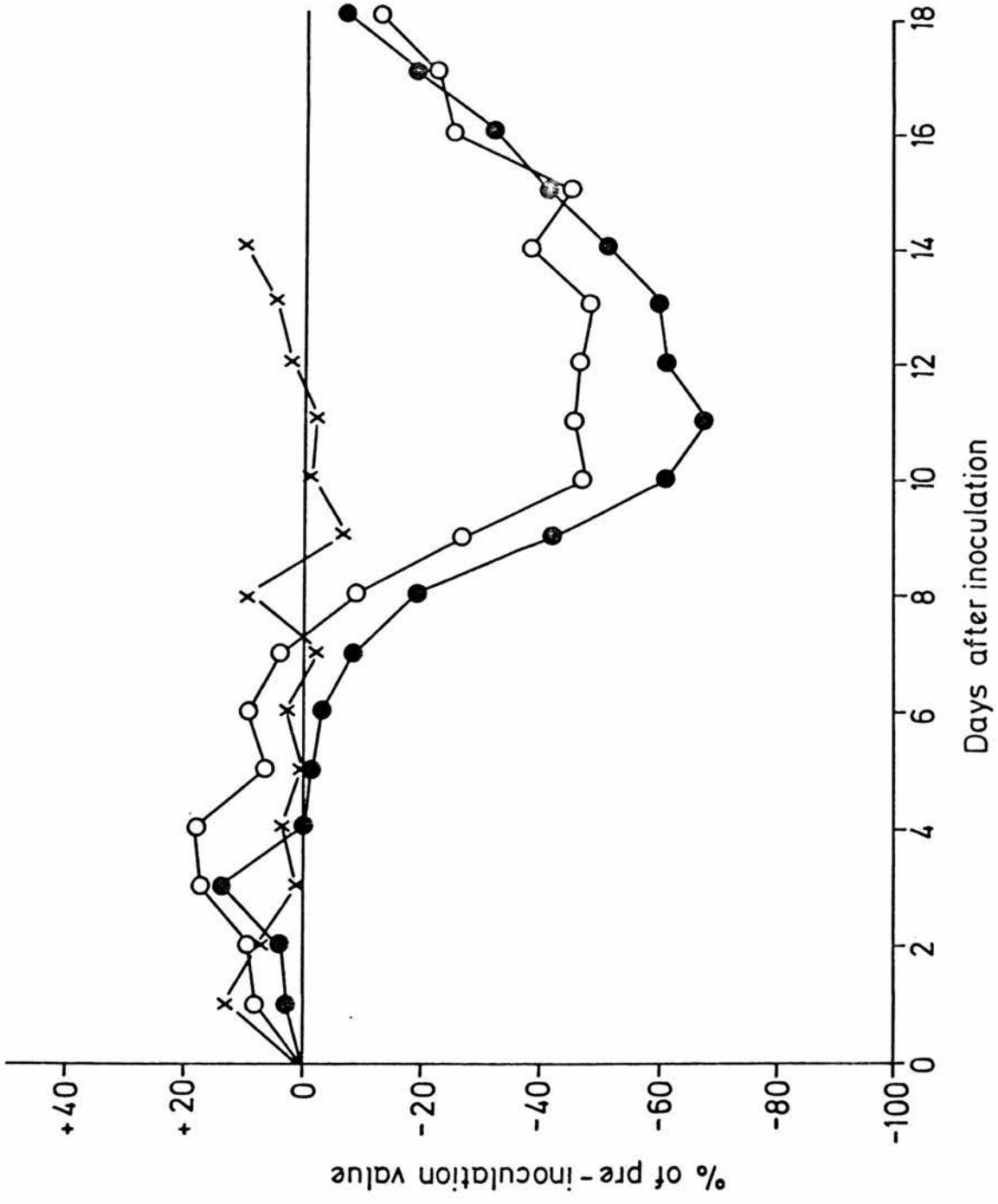

% of pre-inoculation value
+40
+20
0
-20
-40
-60
-80
-100
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.14 Mean daily eosinophil counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

x PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)

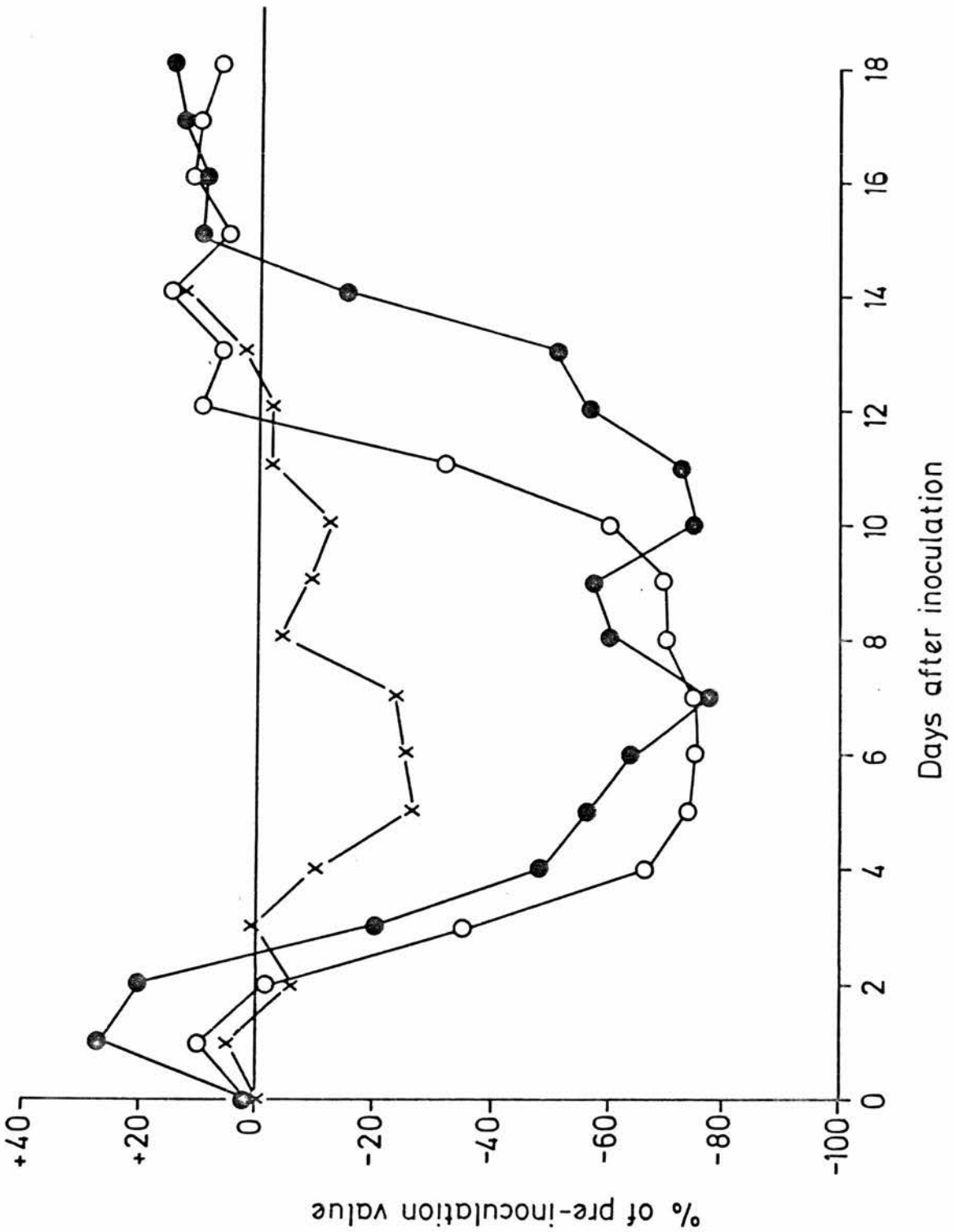

+40
+20
0
-20
-40
-60
-80
-100
% of pre-inoculation value
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.15 Mean daily monocyte counts expressed as percentages of the pre-inoculation value in groups of ten lambs inoculated with PI-3 virus alone, TBF-infected blood 10^{-1} alone, and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

x PI-3 virus alone (Group A)

● TBF 10^{-1} alone (Group E)

o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)

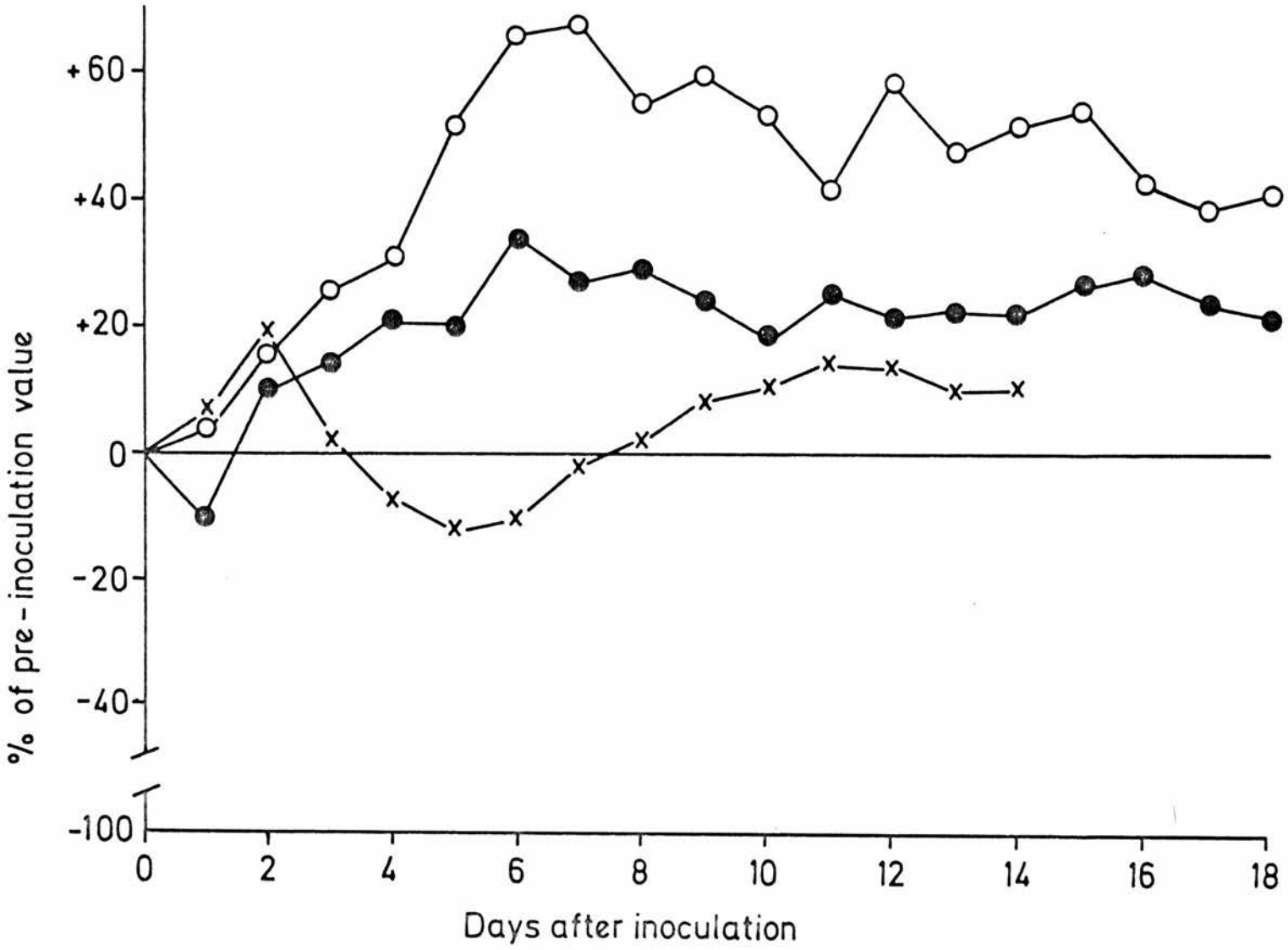

+60
+40
+20
0
-20
-40
-100
% of pre-inoculation value
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.16 Mean parasitaemic reactions in groups of ten lambs inoculated with TBF-infected blood 10^{-1} alone and simultaneously with PI-3 virus and TBF-infected blood 10^{-1}.

● TBF 10^{-1} alone (Group E)

o PI-3 virus and TBF 10^{-1}, simultaneous (Group B)

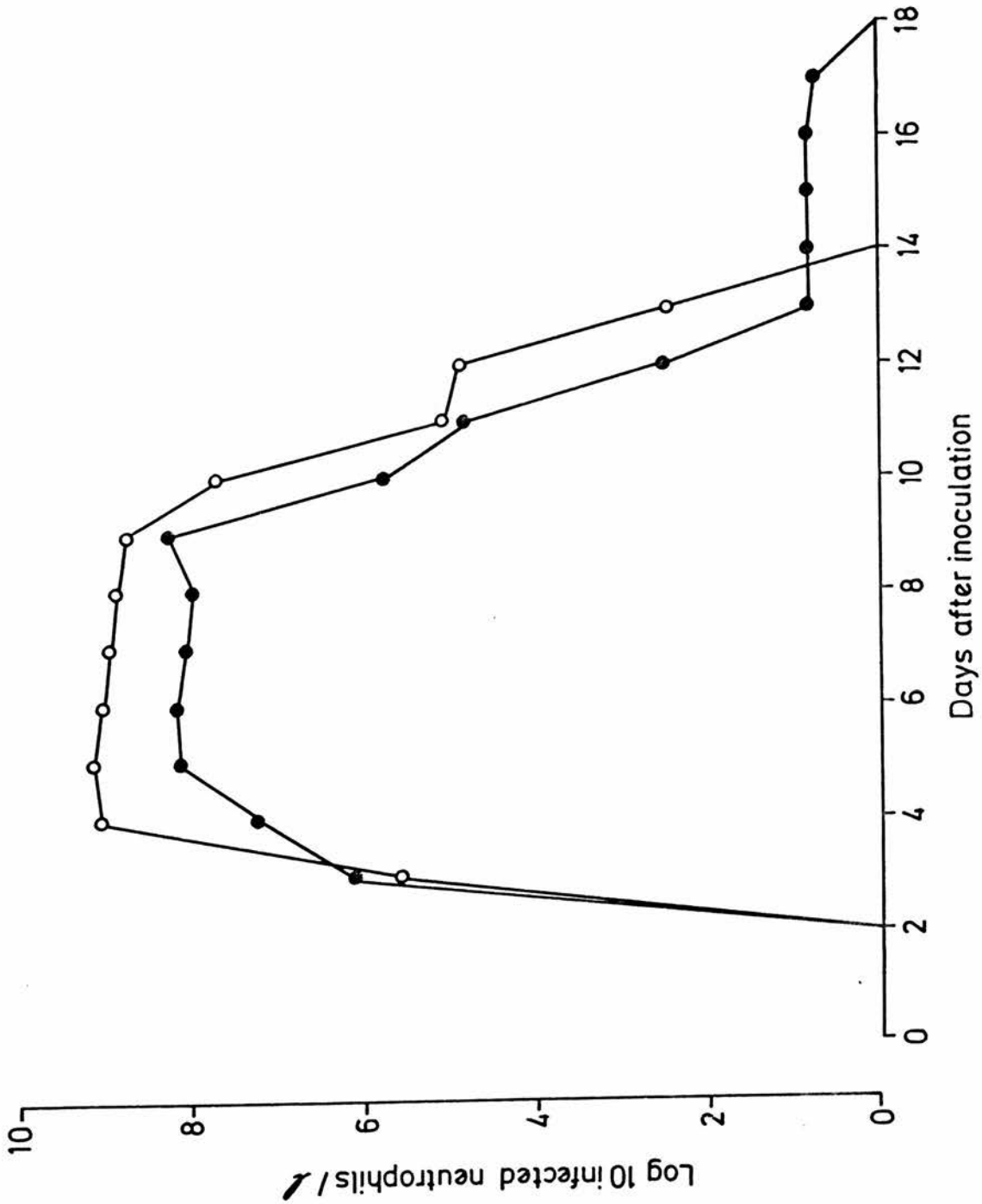

Log 10 infected neutrophils / µl
0
2
4
6
8
10
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.17 Mean parasitaemic reactions in groups of ten lambs inoculated with TBF-infected blood 10^{-1} alone and simultaneously with PI-3 virus and TBF-infected blood 10^{-3}.

- ● TBF 10^{-1} alone (Group E)
- o PI-3 virus and TBF 10^{-3}, simultaneous (Group C)

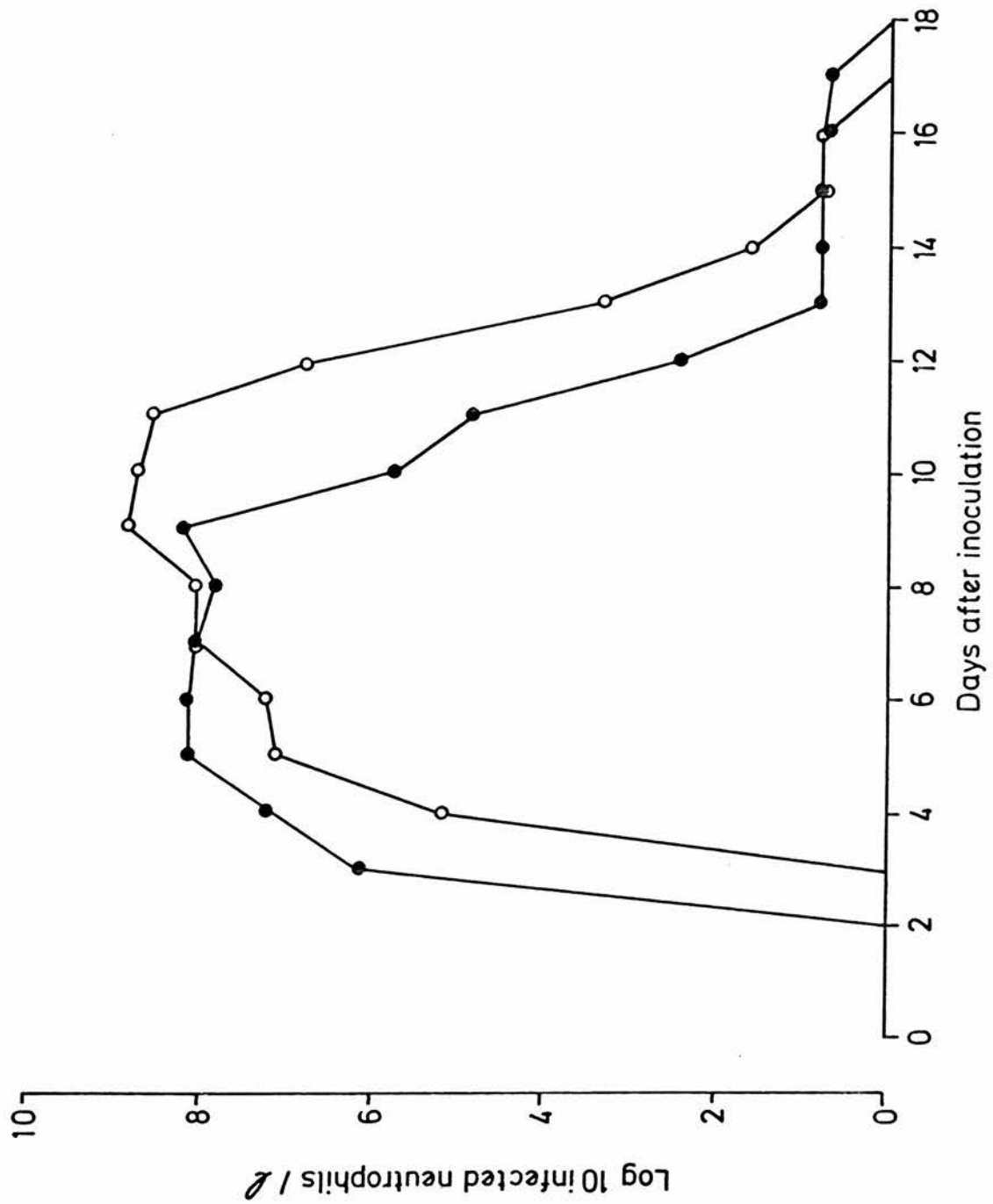
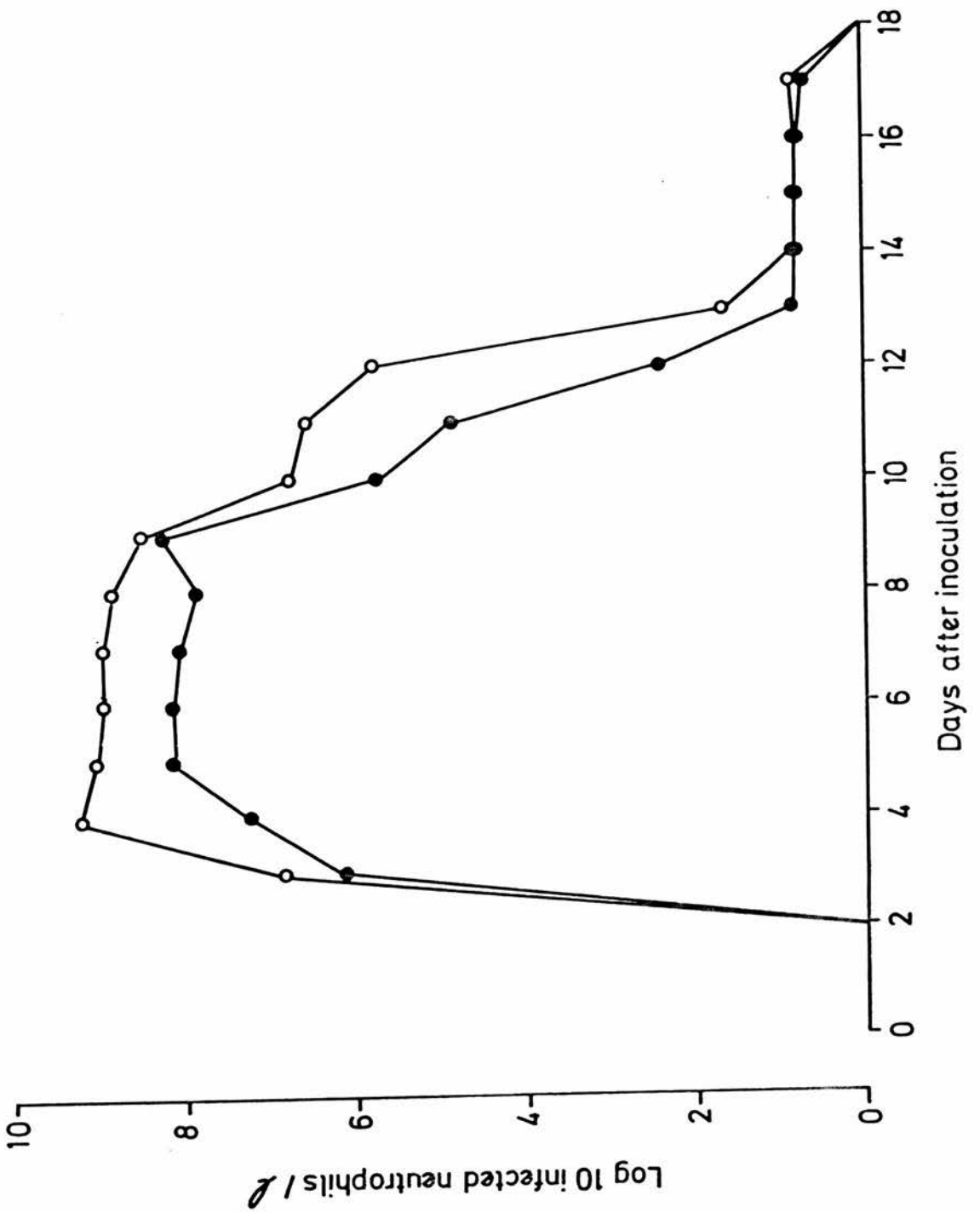

Log 10 infected neutrophils / μl
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
Days after inoculation

Fig. 7.18 Mean parasitaemic reactions in groups of ten lambs inoculated with TBF-infected blood 10^{-1} alone and with TBF-infected blood 10^{-1} and PI-3 virus at the onset of TBF parasitaemia.

- ● TBF 10^{-1} alone (Group E)
- o TBF 10^{-1} and PI-3 virus at the onset of TBF parasitaemia (Group D)


Log 10 infected neutrophils / ℓ
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
Days after inoculation

CHAPTER EIGHT

ATTEMPTS TO PRODUCE TICK PYAEMIA

Tick pyaemia is a staphylococcal infection of lambs prevalent in the tick-infested areas of Britain; the seasonal incidence of the disease being dependent on both the activity of ticks and presence of young lambs (Stewart and Ponsford, 1937; Taylor *et al.*, 1941; Foggie, 1943).

The disease is characterised by the formation of abscesses in the joints and elsewhere. Sometimes it takes the form of a fatal septicaemia (McDiarmid, 1946a). Field cases of tick pyaemia are often complicated with other diseases of lambs such as entero-toxaemia and louping-ill (Stewart and Ponsford, 1937; Taylor *et al.*, 1941; McDiarmid, 1946a). Taylor and his colleagues (1941) noted the frequency of concurrent infection with TBF and drew attention to the possibility of TBF being the predisposing factor to tick pyaemia. Foggie (1956; 1957) later provided the experimental evidence that this was probably so when he showed that the resistance of young lambs to intravenously injected staphylococci was reduced during the TBF-induced neutropaenia.

The present study was designed to examine the effect of concurrent *C. phagocytophila* infection on naturally acquired staphylococcal infection in lambs.

MATERIALS AND METHODS

Animals

Eight pairs of twin lambs showing crops of naturally acquired staphylococcal pustules around the lips and on the perineum were selected from a flock which was regularly examined clinically. The lambs were three to four-weeks-old except for numbers eight and 147 which were eight-weeks-old. The ewes were retained with the lambs in isolation rooms during the period of observation.

Rickettsia

The Old Sourhope strain of *C. phagocytophila* was used. One ml of a 10^{-1} dilution of TBF-infected blood was inoculated intravenously into one of each pair, the other lamb was given intravenously one ml of sterile PBS and served as control.

Observations

The lambs were examined clinically every day. Rectal temperatures were read and blood samples were collected before and after inoculation at 24-hour intervals. The course of the TBF parasitaemia was followed by examining Giemsa-stained blood smears. Total and differential leucocyte counts were carried out as described previously in Chapter Three.

Bacterial culture

Pus from the pustules were inoculated onto blood agar plates and incubated aerobically at 37°C for 24 hours. The plates were examined for colony formation. Gram-stained smears from single colonies were prepared and examined under a light microscope. A

tube test using fresh rabbit plasma was employed to assess the coagulase production of the staphylococcal colonies; each isolate was also inoculated into nutrient gelatin (Cowan, 1974).

RESULTS

Clinical observations

Fever. The TBF-infected lambs had temperatures of over 40.5°C two to four days after inoculation (Table 8.1, Appendix Table 34). The ensuing febrile reaction persisted for three to ten days. A mean maximal temperature of 41.6 ± 0.4°C occurred 1.2 ± 0.8 days after the onset of fever, the mean magnitude of the febrile reaction being 1,718.7 ± 818.6 mm^2 (Table 8.1).

Seven and nine days after the onset of fever, two of the TBF-infected lambs (No. 8 and 147) were observed to be lame. On examination, one of the front feet was found to be slightly swollen and the skin of the interdigital cleft was moist with foul-smelling discharge; there was no under-running of the horn at this stage. The lesion was typical of foot-rot. The durations of the febrile reaction of the two lambs with foot-rot were significantly longer than those of the other TBF-infected lambs ($t_6 = 5.568$, $P < 0.010$) (Table 8.1).

The staphylococcal pustules showed no tendency to spread or to give rise to a generalised infection. They healed up within three weeks.

The pustules on the control siblings healed similarly and the animals otherwise remained clinically normal throughout the

observation period.

Haematology

TBF-infected lambs. The total leucocyte counts dropped progressively and reached a mean nadir of $5.6 \times 10^9 \pm 0.9 \times 10^9$ per l, 4.3 ± 1.5 days after inoculation (Tables 8.2 and 8.3). The leucopaenia however was significant only on days four and five (Table 8.4).

There was a significant decrease in the mean lymphocyte count about the time of onset of visible parasitaemia (Table 8.4) and the count reached a mean nadir of $2.6 \times 10^9 \pm 0.7 \times 10^9$ per l, 4.6 ± 1.0 days after inoculation (Table 8.3). The lymphocytopaenia lasted six days and had a mean magnitude of $2,134 \pm 1,442$ mm^2 (Tables 8.4 and 8.5, Appendix Table 35).

The neutrophil count increased after inoculation, the mean magnitude of the neutrophilia being 702 ± 642 mm^2 (Tables 8.2 and 8.5, Appendix Table 36). The neutrophilia however was not significant and was followed by a sudden drop in the number of neutrophils. The neutrophil count reached a mean nadir of $0.5 \times 10^9 \pm 0.1 \times 10^9$ per l, 6.6 ± 1.1 days after the onset of visible parasitaemia (Table 8.3). The neutropaenic phase lasted at least six days and had a mean magnitude of $3,165 \pm 898$ mm^2 (Tables 8.4 and 8.5, Appendix Table 36).

The number of eosinophils also fell during the reaction but the eosinopaenia was significant only on day six (Tables 8.2 and 8.6). The mean magnitude of the eosinopaenia was $4,015 \pm 992$ mm^2 (Table 8.5, Appendix Table 37).

The number of monocytes increased significantly four days after the onset of visible parasitaemia; the monocytosis lasted three days, the mean magnitude being 3,053.5 ± 1,797.6 mm^2 (Tables 8.2, 8.5 and 8.6, Appendix Table 38).

Control lambs. Changes in the daily total and differential leucocyte counts in the non-infected control lambs following the saline inoculation were minimal (Table 8.7) and non-significant. When the haematological data for the control lambs were compared with those of the infected siblings significant differences were found in the magnitudes of the lymphocytopaenias, neutropaenias, eosinopaenias and monocytosies (Table 8.5, Appendix Tables 39, 40, 41, 42).

Parasitaemia

Characteristic TBF-inclusion bodies were detected in the peripheral blood of the TBF-infected lambs three to five days after inoculation (Table 8.8). The duration of the visible parasitaemia varied from four to eight days, the mean being 6.1 ± 1.3 days (Table 8.8).

A mean maximal parasitaemia of $10^{9.27 \pm 0.2}$ infected neutrophils per l was observed 1.6 ± 0.7 days after the onset of visible parasitaemia (Table 8.8). The mean magnitude of the parasitaemia was 13,629 ± 3,054 mm^2 (Table 8.8).

Bacterial culture

The strains of staphylococci that were isolated from each of the 16 lambs were found to be coagulase-positive. They also liquefied gelatin and formed colonies surrounded by marked zones of

beta-haemolysis on sheep blood agar.

Comparison

Clinical responses. The febrile responses of the six TBF-infected lambs which did not have foot-rot were compared to the responses of the eight TBF-infected adult sheep described in Chapter Three. The mean incubation period was found to be significantly longer in the lambs than in the adult sheep (Table 8.9). Although the mean duration of the fever in the adult sheep was significantly longer than that of the lambs, the mean magnitude of their febrile reactions, mean maximal temperatures and the day on which the maximal temperatures occurred were similar (Table 8.9).

Haematological responses. The mean magnitudes of the haematological parameters in the TBF-infected lambs were similar to those observed in the TBF-infected adult sheep during the 14-day-observation period (Table 8.10).

Parasitaemia. The mean prepatent period was also significantly longer in the lambs than in the adult sheep (Table 8.9). The mean duration of the visible parasitaemia, on the other hand, was longer in the adult sheep; similarly, the mean magnitude of the parasitaemia was significantly greater than in the lambs (Table 8.9). The mean maximal parasitaemias and the day on which they occurred were similar for both groups (Table 8.9).

DISCUSSION

Tick pyaemia was first described by M'Fadyean in 1894 who then attempted to reproduce the disease in lambs by subcutaneous

inoculation of suspensions of the causal organism but succeeded only in producing abscesses at the sites of injection. McDiarmid (1946b; 1948) after having failed to produce pyaemia by intraperitoneal and subcutaneous inoculation of Staphylococcus aureus into guinea pigs, eventually succeeded in producing the acute and pyaemic forms of the staphylococcal infection in guinea pigs and young lambs by the intravenous inoculation of varying doses of the staphylococci.

Taylor and his co-workers (1941) having recognised the possibility of TBF being the predisposing factor to the staphylococcal infections attempted but failed to produce pyaemia by inoculating lambs subcutaneously with S. aureus during the early stage of the febrile reaction to TBF-infection; the resulting local abscess did not give rise to a generalised infection either in the normal or in the TBF-infected lambs. They suspected that infestation with ticks in some way provided an unknown factor favourable to the development of pyaemia following the formation of superficial abscesses. Subsequently, Foggie (1947; 1959) showed that although tick pyaemia is associated with tick-infestation, the tick was unlikely to act as a true vector of the causal staphylococci; he suggested that the tick merely acted as an inoculator of an infection derived from some outside source. Attempts to establish pyaemia in normal and neutropaenic lambs by the introduction of staphylococci into the skin either by intradermal inoculation of tick salivary gland extracts or by allowing ticks to feed on heavily contaminated skin also failed. He suggested that the role of the tick in the aetiology of tick pyaemia was confined to the transmission

of TBF.

Further experiments by Foggie (1956; 1957) on the effect of TBF on the susceptibility of lambs to pyaemia showed that young lambs suffering from a TBF-induced neutropaenia were less resistant to intravenously injected staphylococci than normal lambs because the pyaemia was produced with much smaller numbers of staphylococci in the neutropaenic animals. However, when he infected lambs simultaneously with TBF and S. aureus by the intravenous route increased susceptibility to the bacterial infection was not demonstrated; pyaemia did not occur although the lambs developed TBF. He further demonstrated that intradermal inoculation and single or daily subcutaneous injections of staphylococci into neutropaenic lambs also did not produce pyaemia.

My attempt to induce pyaemia in lambs by superimposing TBF on an already existing naturally acquired staphylococcal infection also failed. My findings however support the hypothesis that pyaemia is not caused by invasion from an already existing superficial lesion, a point which Foggie (1957) claimed to be supported when numerous field cases of the disease were examined and no macroscopic cutaneous or subcutaneous lesions were detected.

The results of previous attempts to produce pyaemia in normal and TBF-infected lambs indicated that the disease was only produced by the intravenous inoculation of the staphylococci and that even in the neutropaenic animals the bacteria have to be introduced directly into the blood stream. Moreover, Foggie (1956; 1957) demonstrated that the time interval between TBF-infection and the

inoculation of the staphylococci was critical in the production of pyaemia. Thus, Taylor and his colleagues (1941) failed to induce pyaemia in the TBF-infected lambs possibly because they not only inoculated the staphylococci subcutaneously but also gave the bacteria at a time before the resistance of the lambs had been reduced by the TBF-infection. Scrutiny of their report revealed that they infected the lambs with the staphylococci four days after the TBF-inoculation which coincided with the period of TBF-induced neutrophilia.

In Easter Bush*, staphylococcal dermatitis occurs when the lambs are three to ten-weeks-old and further attempts to produce pyaemia by exposing the lambs to TBF therefore, should be done before the occurrence of the staphylococcal infection.

Venn and Woodford (1956) reported having observed foot-rot to be one of the complications of tick-borne fever in cattle. In the present study, two out of the eight TBF-infected lambs developed foot-rot seven and nine days after the onset of fever. The clinical reactions of these lambs were found to be more severe than the reactions of the other TBF-infected lambs which did not have foot-rot.

Although the present study failed in its primary objective, it nevertheless provided an opportunity to compare the clinical and haematological responses, and the parasitaemias in the TBF-infected lambs with the responses in the adult sheep. McEwen (1947) observed that under natural conditions, tick-borne fever infections tended to be mild in lambs during the first two-weeks of

*Veterinary Field Station, University of Edinburgh

life. He noted that TBF generally caused only a temperature reaction of from one to six days' duration without other obvious clinical signs. The relatively benign nature of TBF-infection in young animals was also noted by Hudson (1940). He found that a high proportion of calves below six months of age failed to give a febrile reaction to inoculation with blood proved to be infective to older cattle.

The results of the present study confirmed these reports. I found the mean incubation period to be significantly longer and the mean duration of the fever shorter in the lambs than in the adult sheep. Similarly, there were significant differences in the parasitaemias; the mean prepatent period was found to be shorter and the mean duration of the visible parasitaemia longer in the adult sheep than in the lambs. The mean magnitude of the parasitaemias was also less in the lambs. On the other hand, the mean magnitude of the haematological parameters in the lambs were similar to those observed in the adult sheep, a result not hitherto recorded.

Table 8.1 Parameters of the febrile reaction in eight TBF-infected lambs

Sheep No.	Incubation period (days)	Maximal temperature (°C)	Days after the onset of fever	Duration of fever (days)	Magnitude of febrile reaction (mm^2)
8	2	42.2	1	10	3,500
60	4	41.3	3	6	1,775
66	4	41.2	1	6	2,075
82	4	42.0	1	4	1,575
103	4	42.2	1	3	1,375
143	4	41.4	1	3	875
147	3	41.3	2	8	1,575
152	4	41.4	0	3	1,000
mean	3.6	41.6	1.2	5.3	1,718.7
standard deviation	0.7	0.4	0.8	2.6	818.6

Table 8.2 Means of the daily total leucocyte, lymphocyte and neutrophil counts, and median values of the daily eosinophil and monocyte counts in eight TBF-infected lambs

Days after inoculation	Parasitaemia	Total leucocyte count ($\times 10^9/l$)	Lymphocyte count ($\times 10^9/l$)	Neutrophil count ($\times 10^9/l$)	Eosinophil count ($\times 10^9/l$)	Monocyte count ($\times 10^9/l$)
0	-	11.5	8.1	2.8	0.209	0.173
1	-	11.0	7.5	3.0	0.273	0.271
2	-	10.2	6.8	3.1	0.357	0.276
3	+	8.2	4.5	3.0	0.161	0.243
4	+	6.3	3.0	3.0	0.076	0.174
5	+	6.7	2.9	3.3	0.0	0.342
6	+	6.8	3.4	3.2	0.0	0.639
7	+	7.8	3.4	3.4	0.050	0.683
8	+	7.7	4.6	2.0	0.093	0.669
9	+	7.6	5.7	0.9	0.080	0.658
10	+	7.3	5.8	0.7	0.110	0.478
11	+	7.5	5.3	0.5	0.167	0.467
12	-	7.1	5.5	0.8	0.116	0.267
13	-	7.1	5.5	1.1	0.062	0.257
14	-	7.1	5.3	1.3	0.146	0.188

Table 8.3 Total leucocyte count, lymphocyte count and neutrophil count nadirs in eight TBF-infected lambs

Sheep No.	Total leucocyte count nadir ($x10^9/l$)	Days after inoculation	Lymphocyte count nadir ($x10^9/l$)	Days after inoculation	Neutrophil count nadir ($x10^9/l$)	Days after the onset of visible parasitaemia
8	7.3	4	4.2	4	0.6	7
60	4.3	4	1.9	5	0.6	4
66	5.2	8	2.9	7	0.7	6
82	5.3	4	2.5	4	0.5	7
103	6.6	4	2.5	4	0.4	7
143	5.1	4	2.9	4	0.5	7
147	5.6	3	2.1	5	0.3	8
152	4.9	4	2.2	4	0.5	7
mean	5.5	4.3	2.6	4.6	0.5	6.6
standard deviation	0.9	1.5	0.7	1.0	0.1	1.1

Table 8.4 Means and standard errors of differences from pre-inoculation counts of leucocytes, lymphocytes and neutrophils (x 10^9/l) in eight TBF-infected lambs

Days after inoculation	Parasitaemia	Leucocyte	Lymphocyte	Neutrophil
0	-			
1	-	0.4 ± 0.4	0.6 ± 0.4	-0.2 ± 0.1
2	-	1.2 ± 0.7	1.3 ± 0.6	-0.2 ± 0.3
3	+	3.2 ± 1.4	3.5 ± 1.0*	-0.08 ± 0.4
4	+	5.2 ± 2.1*	5.0 ± 1.6*	-0.09 ± 0.3
5	+	4.7 ± 2.0*	5.2 ± 1.3**	-0.4 ± 0.5
6	+	3.7 ± 2.0	4.6 ± 1.4*	-0.4 ± 0.4
7	+	3.6 ± 1.9	4.6 ± 1.5*	-0.6 ± 0.3
8	+	3.8 ± 1.8	3.4 ± 1.3*	0.8 ± 0.4
9	+	3.7 ± 1.7	2.3 ± 1.1	1.8 ± 0.5*
10	+	2.6 ± 1.5	2.2 ± 1.3	2.0 ± 0.4**
11	+	3.8 ± 2.2	2.8 ± 1.6	2.2 ± 0.5**
12	-	4.4 ± 2.2	2.5 ± 1.7	1.8 ± 0.4**
13	-	4.3 ± 2.2	2.6 ± 1.7	1.7 ± 0.4**
14	-	4.3 ± 2.2	2.7 ± 1.7	1.4 ± 0.5*

* $P < 0.050$

** $P < 0.010$

Table 8.5 Haematological parameters of TBF: comparison between eight TBF-infected lambs and the eight non-infected siblings

Parameter	TBF-infected lambs	Non-infected lambs	$t_{(14)}$
Magnitude of lymphocytosis (mm^2)	95.0 ± 145	397 ± 426	1.898
Magnitude of lymphocytopaenia (mm^2)	2,134 ± 1,442	555 ± 667	2.811*
Magnitude of neutrophilia (mm^2)	702 ± 642	723 ± 976	0.050
Magnitude of neutropaenia (mm^2)	3,165 ± 898	365 ± 430	7.954***
Magnitude of eosinophilia (mm^2)	376.8 ± 230.5	1,033 ± 1,049	1.728
Magnitude of eosinopaenia (mm^2)	4,015 ± 992	1,033 ± 1,335	5.071***
Magnitude of monocytopaenia (mm^2)	5,245 ± 600	187 ± 464	1.257
Magnitude of monocytosis (mm^2)	3,053.5 ± 1,797.6	777 ± 1,149	3.018**

* $P < 0.050$

** $P < 0.010$

*** $P < 0.001$

Table 8.6 Means and standard error of differences from pre-inoculation counts of eosinophils and monocytes (x 10^9/l) in eight TBF-infected lambs

Days after inoculation	Parasitaemia	Eosinophil	Monocyte
0	-		
1	-	0.02 ± 0.04	-0.04 ± 0.03
2	-	0.1 ± 0.06	-0.02 ± 0.04
3	+	0.04 ± 0.08	-0.1 ± 0.1
4	+	0.09 ± 0.08	-0.08 ± 0.1
5	+	0.1 ± 0.07	-0.2 ± 0.1
6	+	0.1 ± 0.06*	-0.3 ± 0.2
7	+	0.08 - 0.08	-0.6 ± 0.2*
8	+	0.1 ± 0.05	-0.6 ± 0.2*
9	+	0.1 ± 0.09	-0.4 ± 0.09**
10	+	0.04 ± 0.08	-0.2 ± 0.07
11	+	0.08 ± 0.07	-0.2 ± 0.1
12	-	0.1 ± 0.05	-0.1 ± 0.1
13	-	0.04 ± 0.1	-0.09 ± 0.08
14	-	0.1 ± 0.05	-0.03 ± 0.05

* $P < 0.050$

** $P < 0.010$

Table 8.7 Means of the daily total leucocyte, lymphocyte and neutrophil counts, and median values of the daily eosinophil and monocyte counts in the eight non-infected lambs

Days after inoculation	Total leucocyte count ($x10^9/l$)	Lymphocyte count ($x10^9/l$)	Neutrophil count ($x10^9/l$)	Eosinophil count ($x10^9/l$)	Monocyte count ($x10^9/l$)
0	11.8	7.9	3.0	0.402	0.231
1	11.7	7.5	3.4	0.472	0.251
2	11.6	7.6	3.3	0.426	0.220
3	11.5	7.5	3.3	0.513	0.247
4	11.1	7.1	3.4	0.390	0.166
5	11.5	7.5	3.4	0.384	0.172
6	11.4	7.4	3.3	0.437	0.265
7	11.2	7.3	3.1	0.498	0.211
8	11.3	7.4	3.0	0.466	0.220
9	11.0	7.4	2.9	0.377	0.216
10	11.4	7.8	2.9	0.384	0.225
11	11.2	7.7	2.8	0.360	0.262
12	11.9	8.0	3.4	0.330	0.222
13	11.6	8.2	3.1	0.450	0.282
14	11.0	7.2	3.2	0.345	0.268

Table 8.8 Parameters of the parasitaemia in eight TBF-infected lambs

Sheep No.	Prepatent period (days)	Maximal parasitaemia (log_{10} infected neutrophils/l)	Days after the onset of visible parasitaemia	Duration of visible parasitaemia (days)	Magnitude of parasitaemia (mm^2)
8	3	9.23	3	7	15,735
60	4	9.41	2	8	17,510
66	5	9.55	1	7	15,795
82	4	9.21	1	5	11,040
103	4	9.16	1	5	11,052
143	4	9.11	2	4	8,902
147	3	9.55	2	7	15,897
152	4	8.99	1	6	13,102
mean	3.8	9.27	1.6	6.1	13,629
standard deviation	0.6	0.2	0.7	1.3	3,054

Table 8.9 Clinical and parasitological parameters of TBF: comparison between adult sheep and young lambs

Parameter	Original eight adult sheep	Six lambs	$t_{(12)}$
Incubation period (days)	2.5 ± 0.5	4.0 ± 0.0	7.281***
Maximal temperature (°C)	41.5 ± 0.4	41.6 ± 0.4	0.462
Days of occurrence of maximal temperature	1.7 ± 0.8	1.1 ± 0.9	1.318
Duration of fever (days)	6.5 ± 1.4	4.1 ± 1.4	3.174**
Magnitude of fever (mm^2)	1,871.8 ± 586.6	1,446 ± 458	1.469
Prepatent period (days)	3.0 ± 0.0	4.1 ± 0.4	7.888***
Maximal parasitaemia (log_{10} infected neutrophils/l)	9.20 ± 0.1	9.23 ± 0.2	0.370
Day of occurrence of maximal parasitaemia	1.7 ± 1.9	1.3 ± 0.5	0.500
Duration of visible parasitaemia (days)	7.5 ± 0.7	5.8 ± 1.4	2.998*
Magnitude of parasitaemia (mm^2)	16,403 ± 1,528	12,900 ± 3,241	2.707*

* P 0.050 ** P 0.010 *** P 0.001

Table 8.10 Haematological parameters of TBF: comparison between adult sheep and young lambs

Parameter	Original eight adult sheep	Six lambs	$t_{(12)}$
Magnitude of lymphocytosis (mm^2)	138.5 ± 109.7	124 ± 160	0.201
Magnitude of lymphocytopaenia (mm^2)	2,693 ± 617	2,646 ± 1,602	0.076
Magnitude of neutrophilia (mm^2)	450 ± 385	572 ± 611	0.459
Magnitude of neutropaenia (mm^2)	2,513 ± 892	2,353 ± 976	0.319
Magnitude of eosinopaenia (mm^2)	4,124 ± 577	4,149 ± 1,114	0.063
Magnitude of monocytosis (mm^2)	1,955 ± 1,717.8	2,587 ± 1,706	0.683

CHAPTER NINE

GENERAL DISCUSSION AND CONCLUSION

MacLeod and Gordon (1933) remarked that although the mortality from TBF was low, the disease was nevertheless of considerable economic importance in its debilitating effect and in influencing the course of other infections. They had earlier (1932) shown that TBF triggered the development and aggravated the nervous signs of louping-ill in sheep, an observation that was later confirmed by Taylor, Holman and Gordon (1941).

Tick-borne fever has long been associated with tick pyaemia, and the high incidence of concurrent C. phagocytophila and staphylococcal infections in young lambs had led Taylor and his colleagues (1941) to suspect that the staphylococcal infections in the field were a sequel to the rickettsial infection.

The increased susceptibility of sheep with TBF to secondary bacterial infections has been recognised also by other workers. The lowering of the resistance of sheep to Pasteurella infection by a previous attack of TBF, for example, was noted by Foggie (1951) and Øveras (1972). Foggie (1951) emphasised that TBF, in itself, very rarely killed but the mortality rate in his experimental sheep within three months of having TBF approached 13 percent; most of the deaths were attributed to pneumonia. The present finding that multiplication of Pasteurella haemolytica in the nasal cavities of sheep occurred during the reaction to C. phagocytophila infection also supports the postulated link between TBF and Pasteurella

pneumonia in sheep. Moreover, the results of the study on the effect of TBF on concurrent PI-3 virus infection showed that tick-borne fever aggravated the losses from pneumonia by triggering the development and increasing the severity of the respiratory disease through its potentiating effect on the virus infection. Grønstøl and Ulvund (1977) reported that TBF predisposed sheep to listeriosis and, recently, Grønstøl and Øveras (1980a) demonstrated that TBF exacerbated the clinical reactions of sheep to infection with Listeria monocytogenes.

Infection with the rickettsia, Eperythrozoon ovis, was also shown by Grønstøl and Øveras (1980) to predispose sheep to listeric septicaemia. The combined infection resulted in prolonged illness and unthriftiness; sheep with dual infections had longer febrile reactions but lower antibody titres to L. monocytogenes than the sheep infected with Listeria alone. Earlier, Seamer and his colleagues (1961) investigated the interaction between Eperythrozoon coccoides and LCM virus and found that E. coccoides enhanced the LCM virus infection in a manner similar to that described by Niven and his co-workers (1952) for MHV. Potentiation of the virus infections was evidenced by severe hepatitis and high mortality in the mice with dual infections. Enhancement of the MHV and LCM virus infections by the rickettsia was observed to be greatest when the virus replication coincided with active eperythrozoonosis. (Gledhill et al., 1955; Seamer et al., 1961). The increased susceptibility of the Kupffer cells to MHV was attributed to the increased phagocytosis induced by E. coccoides (Gledhill et al., 1965). The ability of E. coccoides to suppress interferon response in infected mice was

also suggested as a potentially important mechanism contributing to the increased susceptibility of mice infected with E. coccoides to these viruses (Baker et al., 1971).

Increased susceptibility to other diseases is a feature of protozoan infections. Cattle and sheep with trypanosomiasis for example, were noted to be less resistant to bacterial infections than non-infected animals (Parkin and Hornby, 1930; Hull, 1971). Mackenzie and his colleagues (1975) reported that sheep with experimental Trypanosoma congolense infection often succumbed to secondary bacterial pneumonia due to P. haemolytica. They suggested that the increased predisposition of the trypanosome-infected animals to other diseases was possibly due to the immunosuppressive action of trypanosomes. Reid and his colleagues (1979) subsequently, provided substantial experimental evidence that this was probably so when they showed that chronic T. brucei infection markedly decreased the antibody response of mice to louping-ill virus and consequently increased the severity of the clinical reactions of the mice with dual infections to acute louping-ill virus infection. Similarly, Buxton and his co-workers (1980) demonstrated that the ability of mice to cope with an acute infection with louping-ill virus was significantly reduced by a concurrent infection with the protozoan parasite, Toxoplasma gondii. The immunosuppressive effect of T. gondii was also held responsible for the apparent potentiation of the virus infection in the mice with dual infections.

My findings support the hypothesis that the mechanism underlying the increased susceptibility of sheep infected with C. phagocytophila to other diseases is due to a number of factors :

(a) neutropaenia, (b) functional impairment of neutrophils, (c) change in the lymphocyte population and (d) impairment of antibody response to other antigens. Taylor and his colleagues (1941) had earlier shown that the leucopaenia, or more particularly the neutropaenia, was a distinctive feature of the tick-borne fever reaction. Hudson (1950) commented that the effect of TBF on the leucocytes was such that the normal defence mechanisms were likely to be interfered with. In the early studies of the pathogenesis of TBF scant attention was paid to the lymphocytopaenia; instead attention was focussed on the neutropaenia. Foggie (1956; 1957), for example, provided strong experimental evidence that the neutropaenia resulting from C. phagocytophila infection was a factor in the aetiology of tick pyaemia when he demonstrated that the resistance of young lambs to intravenously injected staphylococci was reduced at least one hundred-fold during the neutropaenic phase of the infection. He further showed that the time interval between the TBF-infection and the inoculation of the staphylococci was critical in the production of pyaemia, a point which might explain why the present attempt to induce pyaemia in lambs by superimposing TBF on an already existing naturally acquired staphylococcal infection failed. On the Scottish hill farms, McEwen (1947) found that lambs became infected with C. phagocytophila during the first two weeks of life. Considering the durations of the incubation period, the febrile phase and the neutropaenic phase of TBF, Foggie (1962) estimated that lambs might be expected to be most susceptible to staphylococcal infection when about three weeks old. In a survey of 54 tick pyaemia cases, he found that the mean

age at which the first symptoms of pyaemia appeared was 3.8 weeks ± 1 week. In addition to the TBF-induced neutropaenia, Foster and Cameron (1970a) postulated that the neutropaenia in tick-borne fever was accompanied by some degree of functional impairment of the neutrophils because they observed that diapedesis of neutrophils containing TBF-inclusion bodies was inhibited. In the present study, the phagocytosis of staphylococci was found to be significantly reduced during the TBF parasitaemia and that this reduction was attributable to the limited phagocytic activity of the parasitised neutrophils.

The nature of the lymphocytopaenia induced by TBF in sheep was further explored in my studies of the types of peripheral lymphocyte involved. Using the techniques now avilable for identifying the B- and T- lymphocytes in sheep, it was observed that the lymphocytopaenia in TBF was associated with a significant decrease in the number of cells bearing surface immunoglobulins and only with a small reduction in the number of T-lymphocytes suggesting that the lymphocytopaenia of TBF results from a depletion in the number of circulating B-lymphocytes. The findings support the histological observation that depletion of lymphoid tissues in the spleen and lymph nodes occurred in animals affected with TBF (Hudson, 1950).

The results of the study on the immune responses of normal and TBF-infected sheep to clostridial vaccine confirmed that C. phagocytophila has an immunosuppressive effect in sheep: both primary and secondary antibody responses to the vaccine were significantly depressed in the sheep clinically affected with TBF. In contrast to the suppressed humoral immune response, the cell-mediated response

as measured by a delayed skin hypersensitivity test was not affected suggesting that the immunosuppression in TBF is probably due to the effect of C. phagocytophila on the cells involved in the humoral immune response. Apart from changes in the lymphocyte population, other factors such as disruption of the spleen and lymph node architecture and of lymphocyte recirculation which impair B- and T-cell co-operation (Murray et al., 1974; Moran, de Rivera and Turk, 1973) are possibly involved in the immunosuppression associated with TBF. Regardless of the exact mechanisms involved, the demonstration of immunosuppression in TBF offers another explanation of the increased susceptibility of TBF-infected sheep to other diseases.

Further evidence of pathogen potentiation and immunosuppression by TBF was provided by the findings that inoculation of PI-3 virus at the onset of the TBF parasitaemia resulted in a severe clinical reaction, prolonged virus excretion and mortality. The exacerbation of the respiratory disease and prolonged virus excretion in the sheep with dual infections were associated with a significant depression in the production of antiviral antibodies. The proliferation of P. haemolytica in the nasal cavities as evidenced by the increased isolation rate of the organism from the nasal secretions during the reaction to C. phagocytophila infection is more difficult to explain in terms of suppressed antibody production. However, I observed that the serum antibody titres to specific P. haemolytica serotypes in the TBF-infected sheep that shed the bacteria were lower than the antibody titres in the shedding non-infected sheep.

The enhancement of the severity of PI-3 virus infection in sheep by TBF is of veterinary importance because of the widespread presence of inapparent PI-3 virus infection (Hore, 1969; Karrar, 1977) and the natural occurrence of P. haemolytica in the sheep population (Gilmour, 1978). In the tick-infested areas of Scotland, Pasteurella pneumonia has been reported to be the most common cause of death in sheep (East of Scotland College of Agriculture Annual Report, 1971-1979). Where diagnosis was extended to include tick-borne fever, many of the affected animals were found to be suffering from concurrent infection with C. phagocytophila; evidence of concomitant PI-3 virus infection was found on odd occasions when histological examination of lung tissues from dead sheep were made (Anon., 1971; 1974; 1978).

The result of the present study also showed that immunosuppression is another feature of TBF which might complicate the problems of sheep management in areas where TBF is enzootic by interfering with the response to immunisation. The annual reports of the East of Scotland College of Agriculture, for example, have for years considered clostridial diseases to be the second most common cause of death in sheep. Serious outbreaks were often attributed to the failure on the part of the farmer to vaccinate or administer the full dose of the vaccine, and sometimes to the quality of the vaccine. Since it has been shown in my studies that TBF suppressed the antibody response of sheep to clostridial vaccine, the possibility of TBF being a contributing factor to vaccine-breakdowns should now be considered.

In the tropics where multiple infections are of common occurrence, the possibility remains that the high prevalence of patent infections in the animal population is attributable to concurrent infections with immunosuppressive parasites including rickettsias. Control measures should therefore take into account the possibility of concurrent infections particularly if vaccination is contemplated. It is tempting to suggest that many of the episodes of alleged vaccine-breakdowns may be attributable to a concurrent immunosuppressive parasite.

CONCLUSION

Cytoecetes phagocytophila, the causative agent of tick-borne fever is an effective immunosuppressant and exacerbates concurrent infections. Infection with C. phagocytophila induces a lymphocytopaenia which is associated with a significant decrease in the number of peripheral B-lymphocytes and with a minimal reduction in the number of T-lymphocytes. The B-cell hypo-responsiveness is manifested in the TBF-infected sheep by depressed humoral immune responses and unimpaired cell-mediated response. In addition, the neutrophil numbers and neutrophil function are affected; the neutropaenia is accompanied by a decrease in the phagocytic activity of the parasitised neutrophils.

It is postulated that the observed potentiation of concurrent infections stems from the combined effect of the immunosuppression and neutrophilic malfunction induced by C. phagocytophila.

REFERENCES

ADEGBOYE, D.A. (1978). A review of Mycoplasma-induced immunosuppression. British Veterinary Journal, 134, 556-560.

AMMANN, A.J. and FUDENBERG, H.H. (1976). Immunodeficiency diseases. Basic and Clinical Immunology (Fudenberg, H.H., Stites, D.P., Caldwell, J.L. and Wells, J.D. eds.) pp. 353-354. Lange Medical Publications, California.

ANON. (1971). East of Scotland College of Agriculture, Veterinary Investigation Service, Annual Report; St. Boswells.

ANON. (1974). East of Scotland College of Agriculture, Veterinary Investigation Service, Annual Report: St. Boswells.

ANON. (1978). East of Scotland College of Agriculture, Veterinary Investigation Service, Annual Report: St. Boswells.

ARCHER, R.K. (1965). Haematological Techniques For Use On Animals. pp. 37-73. Blackwell Scientific Publications, Oxford.

ASKONAS, B.A., CORSINI, A.C., CLAYTON, C.E. and OGILVIE, B.M. (1979). Functional depletion of T- and B-memory cells and other lymphoid cell subpopulations during trypanosomiasis. Immunology, 36, 313-321.

BAKER, H.J., CASSELL, G.H. and LINDSEY, J.R. (1971). Research complications due to Haemobartonella and Eperythrozoon infections in experimental animals. American Journal of Pathology, 64, 625-650.

BALDWIN, D.E., MARSHALL, R.G. and WESSMAN, G.E. (1967). Experimental infection of calves with myxovirus parainfluenza 3 and Pasteurella haemolytica. American Journal of Veterinary Research, 28, 1773-1782.

BANKS, K.L. and HENSON, J.B. (1973). Quantitation of immunoglobulin-bearing lymphocytes and lymphocyte response to mitogens in horses persistently infected by equine infectious anaemia virus. Infection and Immunity, 8, 679-682.

BASTEN, A., MILLER, J.F.A.P., SPRENT, J. and PYE, J. (1972). A receptor for antibody on B lymphocytes. I. Method of detection and functional significance. Journal of Experimental Medicine, 135, 610-626.

BAUER, D.C. and STAVITSKY, A.B. (1961). On the different molecular forms of antibody synthesized by rabbits during the early response to a single injection of protein and cellular antigens. Proceedings of the National Academy of Science, 47, 1667.

BHATT, P.N. and JACOBY, R.O. (1976). Genetic resistance to lethal flavivirus encephalitis. II. Effect of immunosuppression. Journal of Infectious Diseases, 134, 166-173.

BIANCO, C., PATRICK, R. and NUSSENZWEIG, V. (1970). A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. Journal of Experimental Medicine, 132, 702-720.

BIBERSTEIN, E.L. and KENNEDY, P.C. (1959). Septicaemic pasteurellosis in lambs. American Journal of Veterinary Research, 20, 94-101.

BIBERSTEIN, E.L. and THOMPSON, D.A. (1965). Type specificity of immunity to Pasteurella haemolytica infection in mice. Journal of Comparative Pathology, 75, 331-337.

BIBERSTEIN, E.L. and THOMPSON, D.A. (1966). Epidemiological studies on Pasteurella haemolytica in sheep. Journal of Comparative Pathology, 76, 83-94.

BIBERSTEIN, E.L., NISBET, D.J. and THOMPSON, D.A. (1967). Experimental pneumonia in sheep. Journal of Comparative Pathology, 81, 338-351.

BIBERSTEIN, E.L., SHREEVE, B.J. and THOMPSON, D.A. (1970). Variation in carrier rates of Pasteurella haemolytica in sheep flocks. Journal of Comparative Pathology, 80, 499-507.

BIBERSTEIN, E.L., SHREEVE, B.J., ANGUS, K.W. and THOMPSON, D.A. (1971). Experimental pneumonia of sheep. Clinical, microbiological and pathological responses to infection with myxovirus parainfluenza 3 and Pasteurella haemolytica. Journal of Comparative Pathology, 81, 339-351.

BINNS, R.M. and SYMONS, D.B.A. (1974). The ontogeny of lymphocyte immunoglobulin determinants and their relationship to antibody production in the fetal pig. Research in Veterinary Science, 16, 260-262.

BOGGS, D.R. and WINKELSTEIN, A. (1976). White Cell Manual. 3rd edition. p. 36. F.A. Davis Company, Philadelphia, PA.

BORELLA, L. (1969). The suppressive effect of Rauscher leukaemia virus on the secondary antibody response of spleen cells cultured in cell-impermeable diffusion chambers. Journal of Immunology, 103, 185-195.

BOWLES, C.A., WHITE, G.S. and LUCAS, D. (1975). Rosette formation by canine peripheral blood lymphocytes. Journal of Immunology, 114, 399-402.

BRANDRETH, S.R. (1978). A study of indirect haemagglutinating antibody in the nasal secretions of goat and sheep nasopharyngeal carriers of Pasteurella haemolytica. M.Sc. thesis, University of Edinburgh.

BRO-JØRGENSEN, K. and VOLKERT, M. (1974). Defects in the immune system of mice infected with lymphocytic choriomeningitis virus. Infection and Immunity, 9, 605-614.

BUCHANAN, R.E. and GIBBONS, N.E. (eds.) (1974). Bergey's Manual of Determinative Bacteriology. 8th edition. pp. 893-895. Williams and Wilkins, Baltimore.

BUDIARSO, I.T. and HARDJOSWORO, S. (1976). Jembrana disease in Bali cattle. Australian Veterinary Journal, 52, 97.

BUXTON, D., REID, H.W. and POW, I. (1979). Immunosuppression in toxoplasmosis: studies in mice with a clostridial vaccine and louping-ill virus vaccine. Journal of Comparative Pathology, 89, 375-379.

BUXTON, D., REID, H.W., FINLAYSON, J. and POW, I. (1980) Immunosuppression in toxoplasmosis: preliminary studies in mice infected with louping-ill virus. Journal of Comparative Pathology, 90, 331-338.

CAMENGA, D.L., NATHANSON, N. and COLE, G.A. (1974). Cyclophosphamide-potentiated West Nile viral encephalitis: relative influence of cellular and humoral factors. Journal of Infectious Diseases, 130, 634-641.

CANTOR, H. and ASOFSKY, R. (1972). Synergy among lymphoid cells mediating the graft-versus-host response. III. Evidence for interaction between two types of thymus-derived cells. Journal of Experimental Medicine, 135, 764-779.

CARTER, R.L., CHESTERMAN, F.C., ROWSON, K.E.K., SALAMAN, M.H. and WEDDERBURN, N. (1970). A new virus of minimal pathogenicity associated with Friend virus. II. Histological changes and immunodepressive effect. International Journal of Cancer, 5, 103-110.

CEGLOWSKI, W.S. and FRIEDMAN, H. (1968). Immunosuppression by leukaemia viruses. I. Effect of Friend disease virus on cellular and humoral hemolysin responses of mice to a primary immunization with sheep erythrocytes. Journal of Immunology, 101, 594-604.

CHEVILLE, N.F. (1967). Studies on the pathogenesis of Gumboro disease in the Bursa of Fabricius, spleen and thymus of the chicken. American Journal of Pathology, 51, 527-539.

CLAYTON, C.E., OGILVIE, B.M. and ASKONAS, B.A. (1979). Trypanosoma brucei infection in nude mice: B-lymphocyte function is suppressed in the absence of T-lymphocytes. Parasite Immunology, 1, 39-48.

CORSINI, A.C., CLAYTON, C.E., ASKONAS, B.A. and OGILVIE, B.M. (1977). Suppressor cells and loss of B-cell potential in mice infected with Trypanosoma brucei. Clinical and Experimental Immunology, 29, 122-131.

COWAN, S.T. (1974). Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd edition. Cambridge University Press, Cambridge.

COX, F.E.G. and WEDDERBURN, N. (1972). Enhancement and prolongation of Babesia microti infections in mice infected with oncogenic viruses. Journal of General Microbiology, 72, 79-85.

CREMER, N.E., TAYLOR, D.O.N. and HAGENS, S.J. (1966). Antibody formation, latency and leukaemia: infection with Maloney virus. Journal of Immunology, 96, 495-508.

CYPESS, R.H., LUBINIECKI, A.S. and HAMMON, W. (1973). Immunosuppression and increased susceptibility to Japanese B encephalitis virus in Trichinella spiralis-infected mice. Proceedings of the Society for Experimental Biology and Medicine, 143, 463-473.

DANSKIN, D. and BURDIN, M.L. (1963). Bovine petechial fever. Veterinary Record, 75, 391-394.

DAUBNEY, R. and HUDSON, J.R. (1931). Nairobi sheep disease. Parasitology, 23, 507-524.

DAVIES, D.H., DUNGWORTH, D.L., HUMPHREYS, S. and JOHNSON, A.L. (1977). Concurrent infection of lambs with parainfluenza virus type 3 and Pasteurella haemolytica. New Zealand Veterinary Journal, 25, 263-265.

DENNIS, R.A., JACOBY, R.O. and GRIESEMER, R.A. (1969). Development of immunity in fetal dogs: effects of thymectomy. American Journal of Veterinary Research, 30, 1517-1522.

DENT, P.B., PETERSON, R.D.A. and GOOD, R.A. (1965). A defect in cellular immunity during the incubation period of passage A leukaemia in C3H mice. Proceedings of the Society for Experimental Biology and Medicine, 119, 869-871.

EAST OF SCOTLAND COLLEGE OF AGRICULTURE, Veterinary Investigation Service, Annual Report. (1971; 1972; 1973; 1974; 1975; 1976; 1977; 1978; 1979). St. Boswells, Perth, Edinburgh.

EDWARDS, C.Q., DEISS, A., COLE, B. and WARD, J.R. (1975). Haematologic changes in chronic arthritis of mice induced by Mycoplasma arthritidis. Proceedings of the Society for Experimental Biology and Medicine, 150, 664-668.

ESCAJADILLO, C. and BINNS, R.M. (1975). Rosette formation with sheep erythrocytes - a possible T-cell marker in the pig. International Archives of Allergy and Applied Immunology, 48, 261-275.

FAHEY, K.J. (1980). Peanut agglutinin: a marker of presumptive T-lymphocytes in sheep. Surgery and Transplantation, 8, 266.

FARAGHER, J.T., ALLAN, W.H. and WYTHE, P.J. (1974). Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease. Veterinary Record, 95, 385-388.

FAUBERT, G.M. and TANNER, C.E. (1971). Trichinella spiralis: inhibition of sheep haemagglutinins in mice. Experimental Parasitology, 30, 120-123.

FAUBERT, G.M. and TANNER, C.E. (1974). The suppression of sheep rosette-forming cells and the inability of mouse bone marrow cells to reconstitute competence after infection with the nematode Trichinella spiralis. Immunology, 27, 501-505.

FAUBERT, G.M. and TANNER, C.E. (1975). Leuco-agglutination and cytotoxicity of the serum of infected mice and of extracts of Trichinella spiralis larvae and the capacity of infected mouse sera to prolong skin allografts. Immunology, 28, 1041-1050.

FAUBERT, G.M. (1976). Depression of the plaque-forming cells to sheep red blood cells by the new-born larvae of Trichinella spiralis. Immunology, 30, 485-489.

FINNEY, D.J. (ed.) (1952). Statistical Methods in Biological Assay. Hafner Publishing Company, New York.

FLOERSHEIM, G.L., HOPFF, W.H., GASSER, M. and BUCHER, K. (1971). Impairment of cell-mediated immune response by Pseudomonas aeroginosa. Clinical and Experimental Immunology, 9, 241-247.

FOGGIE, A. (1943). The prevention of tick pyaemia in lambs with Staphylococcus toxoid. Veterinary Record, 55, 317.

FOGGIE, A. (1947). Studies on the source of the staphylococcal infection found in tick pyaemia of lambs. Journal of Comparative Pathology and Therapeutics, 57, 245-260.

FOGGIE, A. (1951). Studies on the infectious agent of tick-borne fever in sheep. Journal of Pathology and Bacteriology, 63, 1-15.

FOGGIE, A. (1956). The effect of tick-borne fever on the resistance of lambs to staphylococci. Journal of Comparative Pathology and Therapeutics, 66, 278-285.

FOGGIE, A. (1957). Further experiments on the effect of tick-borne fever infection on the susceptibility of lambs to staphylococci. Journal of Comparative Pathology and Therapeutics, 67, 369-377.

FOGGIE, A. (1959). Studies on the relationship of tick-bite to tick pyaemia of lambs. Annals Tropical Medicine and Parasitology, 53, 27-34.

FOGGIE, A. and ALLISON, C.J. (1960). A note on the occurrence of tick-borne fever in cattle in Scotland with comparative studies of bovine and ovine strains of the organism. Veterinary Record, 72, 767-770.

FOGGIE, A. and HOOD, C.S. (1961). Adaptation of the infectious agent of tick-borne fever to guinea pig and mice. Journal of Comparative Pathology and Therapeutics, 71, 414-427.

FOGGIE, A. (1962). Studies on tick-pyaemia and tick-borne fever. Symposia of the Zoological Society of London, 6, 51-60.

FOSTER, W.N.M. and CAMERON, A.E. (1968). Thrombocytopaenia in sheep associated with experimental tick-borne fever infection. Journal of Comparative Pathology, 78, 251-254.

FOSTER, W.N.M. and CAMERON, A.E. (1968a). Aetiology of enzootic staphylococcal infection (tick pyaemia) in lambs. Field investigations into the relationship between tick-borne fever and tick pyaemia. Journal of Comparative Pathology, 78, 243-250.

FOSTER, W.N.M., FOGGIE, A. and NISBET, D.I. (1968). Haemorrhagic enteritis in sheep experimentally infected with tick-borne fever. Journal of Comparative Pathology, 78, 255-258.

FOSTER, W.N.M. and GREIG, J.C. (1969). Isolation of tick-borne fever from feral goats in New Galloway. Veterinary Record, 85, 585-586.

FOSTER, W.N.M. and CAMERON, A.E. (1970a). Observations on the functional integrity of neutrophil leucocytes infected with tick-borne fever. Journal of Comparative Pathology, 80, 487-491.

FOSTER, W.N.M. and CAMERON, A.E. (1970b). Observations on ovine strains of tick-borne fever. Journal of Comparative Pathology, 80, 429-436.

GERSHON, R.K., COHEN, P., HENCIN, R. and LIEBHABER, S.A. (1972). Suppressor T cells. Journal of Immunology, 108, 586-590.

GIAMBRONE, J.J., EIDSON, C.S. and KLEVEN, S.H. (1977). Effect of infectious bursal disease on the response of chickens to Mycoplasma synoviae, Newcastle disease virus and infectious bronchitis virus. American Journal of Veterinary Research, 38, 251-253.

GIAMBRONE, J.J., DONAHOE, J.P., DAWE, D.L. and EIDSON, C.S. (1977). Specific suppression of the Bursa-dependent immune system of chicks with infectious bursal disease. American Journal of Veterinary Research, 38, 581-583.

GIAMBRONE, J.J. (1978). Longterm effects of infectious bursal disease virus infection in young chickens on immunity to Newcastle disease virus. Poultry Science, 57, 1138.

GILMOUR, N.J.L., DRYSDALE, A., STEVENSON, R.G., HORE, D.E. and BROTHERSTON, J.G. (1968). Reaction of young adult sheep to vaccination and infection with myxovirus parainfluenza 3. Journal of Comparative Pathology, 78, 463-468.

GILMOUR, N.J.L., THOMPSON, D.A. and FRAZER, J. (1974). The recovery of Pasteurella haemolytica from the tonsils of adult sheep. Research in Veterinary Science, 17, 413-414.

GILMOUR, N.J.L. (1978). Pasteurellosis in sheep. Veterinary Record, 102, 100-102.

GILMOUR, N.J.L., SHARP, J.M., DONACHIE, W., BURRELLS, C. and FRAZER, J. (1980). Serum antibody response of ewes and their lambs to Pasteurella haemolytica. Veterinary Record, 107, 505-507.

GLEDHILL, A.W., DICK, G.W.A. and NIVEN, J.S.F. (1955). Mouse hepatitis virus and its pathogenic action. Journal of Pathology and Bacteriology, 69, 299-309.

GLEDHILL, A.W., BILBEY, D.L. and NIVEN, J.S.F. (1965). Effect of certain murine pathogens on phagocytic activity. British Journal of Experimental Pathology, 46, 433-442.

GOODWIN, L.G. (1970). The pathology of African trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 64, 797-817.

GOODWIN, L.G., GREEN, D.G., GUY, M.W. and VOLLER, A. (1972). Immunosuppression during trypanosomiasis. British Journal of Experimental Pathology, 53, 40-43.

GORDON, W.S., BROWNLEE, A., WILSON, D.R. and MACLEOD, J. (1932). 'Tick-borne fever'. (A hitherto undescribed disease of sheep). Journal of Comparative Pathology and Therapeutics, 45, 301-309.

GRABAR, P. (1976). The historical background of immunology. Basic and Clinical Immunology (Fudenberg, H.H., Stites, D.P., Caldwell, J.L. and Wells, J.D. eds.) pp. 3-14. Lange Medical Publications, California.

GREEN, G.M. and KASS, E.H. (1964a). The role of the alveolar macrophage in the clearance of bacteria from the lung. Journal of Experimental Medicine, 119, 167-176.

GREEN, G.M. and KASS, E.H. (1964b). Factors influencing the clearance of bacteria by the lungs. Journal of Clinical Investigation, 43, 769-776.

GREENWOOD, B.M., PLAYFAIR, J.H.L. and TORRIGIANI, G. (1971). Immunosuppression in murine malaria. I. General characteristics. Clinical and Experimental Immunology, 8, 467-478.

GREENWOOD, B.M., BRADLEY-MOORE, A.M., PALIT, A. and BRYCESON, A.D.M. (1972). Immunosuppression in children with malaria. Lancet, 1, 169-172.

GREENWOOD, B.M., WHITTLE, H.C. and MOLYNEUX, D.H. (1973). Immunosuppression in Gambian trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67, 846-850.

GREWAL, A.S., ROUSE, B.T. and BABIUK, L.A. (1976). Erythrocyte rosettes - A marker for bovine T cells. Canadian Journal of Comparative Medicine, 40, 298-305.

GREWAL, A.S., ROUSE, B.T. and BABIUK, L.A. (1978). Characterization of surface receptors on bovine leucocytes. International Archives of Allergy and Applied Immunology, 56, 289-300.

GREY, H.M., RABELLINO, E. and PIROFSKY, B. (1971). Immunoglobulins on the surface of lymphocytes. IV. Distribution in hypogammaglobulinemia, cellular immune deficiency and chronic lymphatic leukemia. Journal of Clinical Investigation, 50, 2368-2383.

GREY, C.L. and THOMSON, R.G. (1971). Pasteurella haemolytica in the tracheal air of calves. Canadian Journal of Comparative Medicine, 35, 121-128.

GRIMES, T.M., KING, D.J. and KLEVEN, S.H. (1976). Application of microtitre cell-culture method to characterization of avian adenovirus. Avian Diseases, 20, 299-310.

GRIST, N.R., ROSS, C.A. and BELL, E.J. (1974). Diagnostic Methods in Clinical Virology. 2nd edition. Blackwell Scientific Publications, Oxford.

GRØNSTØL, H. and ULVUND, M.J. (1977). Listeric septicaemia in sheep associated with tick-borne fever (ehrlichiosis ovis). Acta Veterinaria Scandinavica, 18, 575-577.

GRØNSTØL, H. and ØVERAS, J. (1980). Listeriosis in sheep. Eperythrozoon ovis infection used as a model to study predisposing factors. Acta Veterinaria Scandinavica, 21, 523-532.

GRØNSTØL, H. and ØVERAS, J. (1980a). Listeriosis in sheep. Tick-borne fever used as a model to study predisposing factors. Acta Veterinaria Scandinavica, 21, 533-545.

HAIG, D.A. and DANSKIN, D. (1962). The aetiology of bovine petechial fever (Ondiri disease). Research in Veterinary Science, 3, 129-138.

HALE, L.J. (1958). Biological Laboratory Data. p. 16. Methuen and Co. Ltd., London.

HAMDY, A.H., TRAPP, A.L., GALE, C. and KING, N.B. (1963). Experimental transmission of shipping fever in calves. American Journal of Veterinary Research, 24, 287-294.

HEDDLESTONE, K.L., REISINGER, R.C. and WATKO, L.P. (1962). Studies on the transmission and etiology of bovine shipping fever. American Journal of Veterinary Research, 23, 548-553.

HERON, I., POSKITT, D., CAHILL, R.N.P. and TRNKA, Z. (1978). Spontaneous rosette-forming lymphocytes in sheep lymph. A marker for antigen-binding cells. Cellular Immunology, 41, 178-187.

HETRICK, F.M., CHANG, S., BRYNE, R.J. AND HENSEN, P.A. (1963). The combined effect of Pasteurella multocida and myxovirus parainfluenza-3 upon calves. American Journal of Veterinary Research, 24, 939-947.

HINAIDY, H.K. (1973). Zwei neue infektiose Blutkrankheiten des Rindes in Osterreich. Wiener Tierarztliche Monatsschrift, 12, 364-366.

HODGIN, E.C., McGUIRE, T.C., PERRYMAN, L.E. and GRANT, B.D. (1978). Evaluation of delayed hypersensitivity responses in normal horses and immunodeficient foals. American Journal of Veterinary Research, 39, 1161-1167.

HOERLEIN, A.B., MANSFIELD, M.E., ABINANTI, F.E. and HUEBNER, R.J. (1959). Studies of shipping fever of cattle. I. Parainfluenza-3 virus antibodies in feeder calves. Journal of the American Veterinary Medical Association, 135, 153-160.

HOLMBERG, C.A., MANNING, J.S. and OSBURN, B.I. (1976). Feline malignant lymphomas: comparison of morphologic and immunologic characteristics. American Journal of Veterinary Research, 37, 1455-1460.

HOLMBERG, C.A., MANNING, J.S. and OSBURN, B.T. (1977). Malignant lymphoma with B-lymphocyte characteristics in dogs. American Journal of Veterinary Research, 38, 1877-1879.

HOLMES, P.H., MAMMO, E., THOMSON, A., KNIGHT, P.A. and LUCKEN, R. (1974). Immunosuppression in bovine trypanosomiasis. Veterinary Record, 95, 86-87.

HORE, D.E. (1966). Isolation of ovine strains of parainfluenza virus serologically related to type 3. Veterinary Record, 79, 466-467.

HORE, D.E., STEVENSON, R.G., GILMOUR, N.J.L., VANTSIS, J.T. and THOMPSON, D.A. (1968). Isolation of parainfluenza virus from the lungs and nasal passages of sheep showing respiratory disease. Journal of Comparative Pathology, 78, 259-265.

HORE, D.E. (1969). A survey of sheep sera for antibodies to an ovine strain of PI-3 virus. British Veterinary Journal, 125, 311-316.

HORE, D.E. and STEVENSON, R.G. (1969). Respiratory infection of lambs with an ovine strain of parainfluenza virus type 3. Research in Veterinary Science, 10, 342-350.

HOWARD, R.J., NOTKINS, A.L. and MERGENHAGEN, S.E. (1969). Inhibition of cellular immune reaction in mice infected with lactic dehydrogenase virus. Nature, 221, 873-874.

HOWE, D.L., WOODS, G.T. and MARQUIS, G. (1966). Infection of Bighorn sheep (Ovis canadensis) with myxovirus PI-3 and other respiratory viruses. Results of serologic tests and culture of nasal swabs and lung tissues. Bulletin Wildlife Disease Association, 2, 34-37.

HUDSON, J.R. (1950). The recognition of tick-borne fever as a disease of cattle. British Veterinary Journal, 106, 3-17.

HUDSON, K.M., BYNER, C., FREEMAN, J. and TERRY, R.J. (1976). Immunosuppression, high IgM levels and evasion of the immune response in murine trypanosomiasis. Nature, 264, 256-258.

HULDT, G., GARD, S. and OLOVSON, S.G. (1973). Effect of Toxoplasma gondii on the thymus. Nature, 244, 301-303.

HULL, R.M. (1971). Laboratory studies on a South American strain of Trypanosoma vivax. Transactions of the Royal Society of Tropical Medicine and Hygiene, 65, 258.

JACOBSON, E.D., HERZENBERG, L.A., RIBLET, R. and HERZENBERG, L.A. (1972). Active suppression of immunoglobulin allotype synthesis. II. Transfer of suppressing factor with spleen cells. Journal of Experimental Medicine, 135, 1163-1176.

JAKAB, G.J. and GREEN, G.M. (1972). The effect of Sendai virus infection on bactericidal and transport mechanisms of the murine lung. Journal of Clinical Investigation, 51, 1989-1998.

JAKAB, G.J. and DICK, E.C. (1973). Synergistic effect of viral-bacterial infection: combined infection of the murine respiratory tract with Sendai virus and Pasteurella pneumotropica. Infection and Immunity, 8, 762-768.

JAMIESON, S. (1947). Some aspects of immunity to tick-borne fever in hoggs. Veterinary Record, 59, 202-202.

JAMIESON, S. (1950). Tick-borne fever as a cause of abortion in sheep - Part II. Veterinary Record, 62, 468-470.

JANOSSY, G. and GREAVES, M.F. (1971). Lymphocyte activation. I. Response of T and B lymphocytes to phytomitogens. Clinical and Experimental Immunology, 9, 483-498.

JAYAWARDENA, A.N. and WAKSMAN, B.H. (1977). Suppressor cells in experimental trypanosomiasis. Nature, 265,539-541.

JERRELLS, T.R., DEAN, S.H., RICHARDSON, G.L. and HERBERMAN, R.B. (1980). Depletion of monocytes from human peripheral blood mononuclear leucocytes: comparison of the Sephadex G-10 column method with other commonly used techniques. Journal of Immunological Methods, 32, 11-29.

JONDAL, M., HOLM, G. and WIGZELL, H. (1972). Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. Journal of Experimental Medicine, 136, 207-215.

KAKLAMANIS, E. and PAVLATOS, M. (1972). The immunosuppressive effect of Mycoplasma infection. I. Effect on the humoral and cellular response. Immunology, 22, 695-702.

KANTZLER, G.B., LAUTERIA, S.F., CUSUMANO, C.L., LEE, J.D., GANGULY, R. and WALDMAN, R.H. (1974). Immunosuppression during influenza virus infection. Infection and Immunity, 10, 996-1002.

KARRAR, A.R.E. (1977). Prevalence of parainfluenza antibodies in sheep. M.Sc. Thesis, University of Edinburgh.

KAYE, D., MERSELIS, J.G. and HOOK, E.W. (1965). Influence of Plasmodium berghei infection on susceptibility to Salmonella infection. Proceedings of the Society for Experimental Biology and Medicine, 120, 810-813.

KIPATOVSKII, I.D. and STANISLAVSKI, E.S. (1971). Immunosuppressive effect of cell-free extracts from Escherichia coli. Transplantation Proceedings, 3, 831-834.

KLOSTERGAARD, J., LISAFELD, B.A., DUNLAP, B., KLEIN, E. and HOLTERMANN, O.A. (1978). Activation of neutrophil granulocytes by products of human lymphocytes. International Archives of Allergy and Applied Immunology, 57, 542-548.

KRETTLI, A.U. and NUSSENZWEIG, R. (1974). Depletion of T and B lymphocytes during malarial infections. Cellular Immunology, 13, 440-446.

KUIL, H., MOLENKAMP, G.J., MEYER, J.C. and MEYER, P. (1977). Tick-borne fever in cattle in the Netherlands. Netherlands Journal of Veterinary Science, 5, 61-63.

LELCHUK, R., TAVERNE, J., AGOMO, P.U. and PLAYFAIR, J.H.L. (1979). Development and suppression of a population of late-adhering macrophages in mouse malaria. Parasite Immunology, 1, 61-78.

LITTLEJOHN, A.I. (1950). Tick-borne fever as a cause of abortion in sheep. Veterinary Record, 62, 577-579.

LIU, C., JAYANETA, P., VOTH, D.W., MUANGMANEE, L. and CHO, C.T. (1972). Potentiating effect of Mycoplasma pneumoniae infection on the development of pneumococcal septicaemia in hamsters. Journal of Infectious Diseases, 125, 603-612.

LOOSE, L.A., COOK, J.A. and DILUZIO, N.R. (1972). Malarial immunosuppression - a macrophage-mediated defect. Proceedings of the Helminthological Society of Washington, 39, 484-491.

LOPEZ, A., THOMSON, R.G. and SAVAN, M. (1976). The pulmonary clearance of Pasteurella haemolytica in calves infected with bovine parainfluenza-3 virus. Canadian Journal of Comparative Medicine, 40, 385-391.

MACKENZIE, P.K.I., BOYT, W.P., EMSLIE, V.W., LANDER, K.P. and SWANEPOEL, R. (1975). Immunosuppression in ovine trypanosomiasis. Veterinary Record, 97, 452-453.

MACLEOD, J. (1932). Preliminary studies in the tick transmission of louping-ill. I. Review and analysis of previous transmission experiments. II. A study of the reaction of sheep to tick infestations. Veterinary Journal, 88, 276-284.

MACLEOD, J. and GORDON, W.S. (1932). Studies in louping-ill: an encephalomyelitis of sheep. II. Transmission by the sheep tick, Ixodes ricinus L. Journal of Comparative Pathology and Therapeutics, 45, 240-256.

MACLEOD, J. and GORDON, W.S. (1933). Studies on the tick-borne fever of sheep. I. Transmission by the tick, Ixodes ricinus, with a description of the disease produced. Parasitology, 25, 273-283.

MACLEOD, J. (1936). Studies on tick-borne fever of sheep. II. Experiments on transmission and distribution of the disease. Parasitology, 28, 320-329.

MACLEOD, J. (1962). Ticks and disease in domestic stock in Great Britain. Symposia of the Zoological Society of London, 6, 29-50.

MALAKIAN, A.H. and SCHWAB, J.H. (1968). Immunosuppressant from group A streptococci. Science, 159, 880-881.

MALAKIAN, A.H. and SCHWAB, J.H. (1971). Biological characterization of an immunosuppressant from group A streptococci. Journal of Experimental Medicine, 134, 1253-1265.

McDIARMID, A. (1946a). Studies on enzootic staphylococcal infection in lambs associated with tick-bite. I. The nature of the disease, clinical findings and differential diagnosis. Veterinary Record, 58, 103-104.

McDIARMID, A. (1946b). Studies on enzootic staphylococcal infection in lambs associated with tick-bite. III. Pathogenicity of the causal organism in the guinea pig. Veterinary Record, 58, 431-432.

McDIARMID, A. (1948). Studies on enzootic staphylococcal infection in lambs associated with tick-bite. IV. Pathogenicity of the causal organism in the lamb. Veterinary Record, 60, 1-3.

McDIARMID, A. (1965). Modern trends in animal health and husbandry. Some infectious diseases of free-living wildlife. British Veterinary Journal, 121, 245-257.

McEWEN, A.D. (1947). Tick-borne fever in young lambs. Veterinary Record, 59, 198-201.

McFARLAND, H.F. (1974). The effect of measles virus infection on T and B lymphocytes in the mouse. I. Suppression of helper cell activity. Journal of Immunology, 113, 1978-1983.

McGREGOR, I.A. and BARR, M. (1962). Antibody response to tetanus toxoid inoculation in malarious and non-malarious Gambian children. Transactions of the Royal Society of Tropical Medicine and Hygiene, 56, 364-367.

MELLA, B. and LANG, D.J. (1967). Leukocyte mitosis: suppression in vitro associated with acute infectious hepatitis. Science, 155, 80-81.

M'FADYEAN, J. (1894). Louping-ill in sheep. Journal of Comparative Pathology and Therapeutics, 7, 207-219.

MIMS, C.A. and WAINWRIGHT, S. (1968). The immunodepressive action of lymphocytic choriomeningitis virus in mice. Journal of Immunology, 101, 717-724.

MORAN, C.J., DE RIVERA, V.S. and TURK, J.L. (1973). The immunological significance of histological changes in the spleen and liver in mouse malaria. Clinical and Experimental Immunology, 13, 467-478.

MORGAN, J.E. and BEESON, P.B. (1971). Experimental observations on the eosinopaenia induced by acute infection. British Journal of Experimental Pathology, 52, 214-220.

MOULTON, J.E. and COLEMAN, J.L. (1977). Immunosuppression in deer mice with experimentally induced trypanosomiasis. American Journal of Veterinary Research, 38, 573-579.

MURRAY, P.K., JENNINGS, F.W., MURRAY, M. and URQUHART, G.M. (1974). The nature of immunosuppression in Trypanosoma brucei infections in mice. II. The role of the T and B lymphocytes. Immunology, 27, 825-840.

MUSCOPLAT, C.C., JOHNSON, D.W. and TEUSCHER, E. (1973). Surface immunoglobulin of circulating lymphocytes in chronic bovine diarrhea: abnormalities in cell populations and cell function. American Journal of Veterinary Research, 34, 1101-1104.

MUSCOPLAT, C.C., JOHNSON, D.W., POMEROY, K.A., OLSON, J.M., LARSON, V.L., STEVENS, J.B. and SORENSEN, D.K. (1974). Lymphocyte subpopulations and immunodeficiency in calves with acute lymphocytic leukemia. American Journal of Veterinary Research, 35, 1571-1573.

NEWBOULD, F.H.S. (1976). Phagocytic activity of bovine leukocytes during pregnancy. Canadian Journal of Comparative Medicine, 40, 111-116.

NIKLASSON, P.M. and WILLIAMS, R.C. (1974). Studies of peripheral blood T- and B-lymphocytes in acute infections. Infection and Immunity, 9, 1-7.

NIVEN, J.S.F., DICK, G.W.A., GLEDHILL, A.W. and ANDREWS, C.H. (1952). Further light on mouse hepatitis. Lancet, ii, 1061.

NOSSAL, G.J.V., AUSTIN, C.M. and ADA, G.L. (1965). Antigens in immunity. VII. Analysis of immunological memory. Immunology, 9, 333-348.

NOTKINS, A.L., MERGENHAGEN, S.E. and HOWARD, R.J. (1970). Effect of virus infections on the function of the immune system. Annual Review of Microbiology, 24, 525-538.

OKADA, T., ISHII, H., YAMAURA, K. and YAMAMOTO, T. (1966). Inhibitory effect of Friend leukemia virus infection on the antibody formation to sheep erythrocytes in mice. Japanese Journal of Experimental Medicine, 36, 277-290.

OLSON, G.B., DENT, P.B., RAWLS, W.E., SOUTH, M.A., MONTGOMERY, J.R., MELNICK, J.L. and GOOD, R.A. (1968). Abnormalities of *in vitro* lymphocyte responses during rubella virus infections. Journal of Experimental Medicine, 128, 47-68.

OPPENHEIM, J.J. (1968). Relationship of *in vitro* lymphocyte transformation to delayed hypersensitivity in guinea pigs and man. Federation Proceedings, 27, 21-27.

OSBALDISTON, G.W. (1973). Laboratory Procedures in Clinical Veterinary Bacteriology. University Park Press, Baltimore.

ØVERAS, J. (1959). Tick-borne fever (sjodogg) as a cause of abortion in ewes. Nordisk Veterinarmedicin, 11, 474-482.

ØVERAS, J. (1962). Studies on immunity to tick-borne fever in sheep in Norway. Nordisk Veterinarmedicin, 14, 620-630.

ØVERAS, J. (1972). Studies on immunity to tick-borne fever. Diseases in sheep kept on *Ixodes ricinus* infected pastures. Nordisk Veterinarmedicin, 83, 561-567.

PARKIN, B.S. and HORNBY, H.E. (1930). A contribution to the study of 'immunity' in bovine trypanosomiasis. 16th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa, 11-20.

PATTISON, M. and ALLAN, W.H. (1974). Infection of chicks with infectious bursal disease and its effect on the carrier state with Newcastle disease virus. Veterinary Record, 95, 65-66.

PEARSON, T.W., ROELANTS, G.E., LUNDIN, L.B. and MAYOR-WITHEY, K.S. (1979). The bovine lymphoid system: binding and stimulation of peripheral blood lymphocytes by lectins. Journal of Immunological Methods, 26, 271-282.

PERNIS, B., FORNI, L. and AMANTES, L. (1970). Immunoglobulin spots on the surface of rabbit lymphocytes. Journal of Experimental Medicine, 132, 1001-1018.

PERRYMAN, L.E. (1979). Primary and secondary immune deficiencies of domestic animals. Advances in Veterinary Science and Comparative Medicine, 23, 23-52.

PHILLIPS, W.A., SHELTON, M.J. and HOSKING, C.S. (1979). A simple micro-assay for neutrophil bactericidal activity. Journal of Immunological Methods, 26, 187-191.

PLAYFAIR, J.H.L. (1971). Cell co-operation in the immune response. Clinical and Experimental Immunology, 8, 839-856.

PURNELL, R.E., YOUNG, E.R., BROCKLESBY, D.W. and HENDRY, D.J. (1977). The haematology of experimentally-induced B. divergens and E. phagocytophila infections in splenectomised calves. Veterinary Record, 100, 4-6.

RABELLINO, E. and GREY, H.M. (1971). Immunoglobulins on the surface of lymphocytes. III. Bursal origin of surce immunoglobulins on chicken lymphocytes. Journal of Immunology, 106, 1418-1420.

RABELLINO, E., COLON, S., GREY, H.M. and UNANUE, E.R. (1971). Immunoglobulins on the surface of lymphocytes. I. Distribution and quantitation. Journal of Experimental Medicine, 133, 156-157.

RAFF, M.C. (1969). Theta isoantigen as a marker of thymus-derived lymphocytes in mice. Nature, 224, 378-379.

RAFF, M.C., STERNBERG, M. and TAYLOR, R.B. (1970). Immunoglobulin determinants on the surface of mouse lymphoid cells. Nature, 225,553-554.

RAFF, M.C. and WORTIS, H.H. (1970). Thymus dependence of theta-bearing cells in the peripheral lymphoid tissues of mice. Immunology, 18, 931-942.

RAFF, M.C. (1973). T and B lymphocytes and immune responses. Nature, 242, 19-23.

REEVES, J.H. and RENSHAW, H.W. (1978). Surface membrane markers on bovine peripheral blood lymphocytes. American Journal of Veterinary Research, 39, 917-923.

REID, H.W., HOLMES, P.H. and SKINNER, H.H. (1979). Immunosuppression in experimental trypanosomiasis: effects of Trypanosoma brucei on immunization against louping-ill virus and lymphocytic choriomeningitis virus. Journal of Comparative Pathology, 89, 581-585.

REID, H.W., BUXTON, D., FINLAYSON, J. and HOLMES, P.H. (1979a). Effect of chronic Trypanosoma brucei infection on the course of louping-ill virus infection in mice. Infection and Immunity, 23, 192-196.

REID, H.W., BUXTON, D., POW, I. and FINLAYSON, J. (1980). Immunosuppression in toxoplasmosis: further studies on mice infected with louping-ill virus. Journal of Medical Microbiology, 13, 313-318.

RETIEF, G.P., NEITZ, W.O. and McFARLANE, I.S. (1971). Observations on the effect of tick-borne fever (Cytoecetes phagocytophila Foggie, 1949) on the spermatogenesis of bulls. Journal of the South African Veterinary Medical Association, 42, 321-325.

ROBBINS, J.B., KENNY, K. and SUTER, E. (1965). The isolation and biological activities of rabbit gamma-M and gamma-G anti-Salmonella typhimurium antibodies. Journal of Experimental Medicine, 122, 385-402.

ROBERTS, D.H., WINDSOR, R.S., MASIGA, W.N. and KARIAVU, C.G. (1973). Cell-mediated immune response in cattle to Mycoplasma mycoides var. mycoides. Infection and Immunity, 8, 349-354.

ROCKLIN, R.E. (1976). Mediators of cellular immunity. Basic and Clinical Immunology (Fudenberg, H.S., Stites, D.P., Caldwell, J.L. and Wells, J.D. eds.) pp. 102-114. Lange Medical Publications, California.

ROITT, I.M., GREAVES, M.F., TORRIGIANI, G., BROSTOFF, J. and PLAYFAIR, J.H.L. (1969). The cellular basis of immunological responses. Lancet, 2, 367-371.

SALAMAN, M.H. and WEDDERBURN, N. (1966). The immunodepressive effect of Friend virus. Immunology, 10, 445-458.

SALAMAN, M.H., WEDDERBURN, N. and BRUCE-CHWATT, L.J. (1969). The immunodepressive effect of a murine plasmodium and its interaction with murine oncogenic viruses. Journal of General Microbiology, 59, 383-391.

SAUNDERS, J.R. and BERMAN, D.T. (1964). Epizootiologic studies of shipping fever of cattle. II. Exposure of calves to Pasteurellae and parainfluenza 3 virus. Canadian Journal of Comparative Medicine, 28, 57-62.

SCHALM, O.W., JAIN, N.C. and CARROLL, E.J. (1975). Veterinary Hematology. 3rd edition. p. 505. Lea and Febiger, Philadelphia.

SCHWAB, J.H. (1975). Suppression of the immune response by micro-organisms. Bacteriological Review, 29, 121-143.

SCOTT, J.M., PEGRAM, R.G., HOLMES, P.H., PAY, T.W.F., KNIGHT, P.A., JENNINGS, F.W. and URQUHART, G.M. (1977). Immunosuppression in bovine trypanosomiasis: field studies using foot-and-mouth disease vaccine and clostridial vaccine. Tropical Animal Health and Production, 9, 159-165.

SEAMER, J., GLEDHILL, A.W., BARLOW, J.L. and HOTCHIN, J. (1961). Effect of Eperythrozoon coccoides upon lymphocytic choriomeningitis in mice. Journal of Immunology, 86, 512-515.

SELL, S. and GELL, P.G.H. (1965). Studies on rabbit lymphocytes in vitro. I. Stimulation of blast transformation with an anti-allotype serum. Journal of Experimental Medicine, 122, 423-437.

SHARP, J.M., GILMOUR, N.J.L., THOMPSON, D.A. and RUSHTON, B. (1978). Experimental infection of specific pathogen-free lambs with parainfluenza virus type 3 and Pasteurella haemolytica. Journal of Comparative Pathology, 88, 237-243.

SHIRAI, A., DIETEL, J.W. and OSTERMAN, J.V. (1975). Indirect haemagglutination test for human antibody to typhus and spotted fever group Rickettsiae. Journal of Clinical Microbiology, 2, 430-437.

SHREEVE, B.J. and THOMPSON, D.A. (1970). Studies on the carriage of Pasteurella haemolytica in lambs. Journal of Comparative Pathology, 80, 107-112.

SIEGEL, B.V. and MORTON, J.I. (1966). Serum agglutinin levels to sheep red blood cells in mice infected with Rauscher virus. Proceedings of the Society for Experimental Biology and Medicine, 183, 467-470.

SINGH, K.V. and ATA, F.A. (1967). Presence of antibodies against PI-3 virus in camel and sheep sera. Veterinary Record, 81, 84.

SMITH, G.R. (1961). Further observations on the pathogenicity of Pasteurella haemolytica for young lambs. Journal of Pathology and Bacteriology, 71, 94-99.

SMITH, G.R. (1964). Production of pneumonia in adult sheep with cultures of Pasteurella haemolytica type A. Journal of Comparative Pathology and Thereapeutics, 74, 241-249.

SMITH, W.D. (1975). The nasal secretion and serum antibody response of lambs following vaccination and aerosol challenge with parainfluenza 3 virus. Research in Veterinary Science, 19, 56-62.

SNEDECOR, G.W. and COCHRAN, W.G. (1967). Statistical Methods. 6th edition. pp. 67-90. Iowa State University Press, Ames, Iowa, U.S.A.

SNODGRASS, D.R. and RAMACHANDRAN, S.A. (1971). A complement-fixation test for tick-borne fever of sheep. British Veterinary Journal, 127, xliv-xlvi.

SNODGRASS, D.R. (1974). Studies on bovine petechial fever and ovine tick-borne fever. Ph.D. Thesis, University of Edinburgh.

SNODGRASS, D.R., KARSTAD, L.H. and COOPER, J.E. (1975). The role of wild ruminants in the epidemiology of bovine petechial fever. Journal of Hygiene, Cambridge, 74, 245-250.

SNODGRASS, D.R. (1975). Pathogenesis of bovine petechial fever. Latent infections, immunity and tissue distribution of Cytoecetes ondiri. Journal of Comparative Pathology, 85, 523-530.

SOLLOD, A.E. and FRANK, G.H. (1979). Bovine trypanosomiasis: effect on the immune response of the host. American Journal of Veterinary Research, 40, 658-664.

STAMP, J.T. and WATT, J.A. (1950). Tick-borne fever as a cause of abortion in sheep - Part I. Veterinary Record, 62, 465-468.

STAMP, J.T., WATT, J.A. and THOMLINSON, J.R. (1955). Pasteurella haemolytica septicaemia of lambs. Journal of Comparative Pathology and Therapeutics, 65, 183-196.

STEWART, W.L. and PONSFORD, A.P. (1937). Lamb disease coincident with louping-ill. Journal of Comparative Pathology and Therapeutics, 50, 395-400.

STITES, D.P. (1976). Laboratory Methods of detecting cellular immune function. Basic and Clinical Immunology (Fudenberg, H.H., Stites, D.P., Caldwell, J.L. and Wells, J.D. eds.) pp. 325-327. Lange Medical Publications, California.

STRICKLAND, G.T., AHMED, A. and SELL, K.W. (1975). Blastogenic response of Toxoplasma-infected mouse spleen cells to T- and B-cell mitogens. Clinical and Experimental Immunology, 22, 167-176.

STRICKLAND, G.T. and SAYLES, P.C. (1977). Depressed antibody responses to a thymus-dependent antigen in toxoplasmosis. Infection and Immunity, 15, 184-190.

SULLIVAN, J.L.D., BARRY, D.W., ALBRECHT, P. and LUCAS, S.J. (1975). Inhibition of lymphocyte stimulation by measles virus. Journal of Immunology, 114, 1458-1461.

SUTER, E. (1956). Interaction between phagocytes and pathogenic micro-organisms. Bacteriological Reviews, 20, 94-132.

SYMONS, D.B.S. and BINNS, R.M. (1975). Immunoglobulin-bearing lymphocytes: their demonstration in adult sheep and ontogeny in the sheep fetus. International Archives of Allergy and Applied Immunology, 49, 658-669.

TAKASHIMA, I. and OLSON, C. (1980). Bovine leukosis virus in sheep, lymphocyte modification and surface immunoglobulin-bearing cell numbers. Veterinary Microbiology, 5, 1-12.

TANABE, K., WAKI, S., TAKADA, S. and SUZUKI, M. (1977). Plasmodium berghei: suppressed response of antibody-forming cells in infected mice. Experimental Parasitology, 43, 143-152.

TAYLOR, A.W., HOLMAN, H.H. and GORDON, W.S. (1941). Attempts to reproduce the pyaemia associated with tick-bite. Part I. The nature of the infections produced. Part II. Comparison of the blood picture of the different infections. Veterinary Record, 53, 337-344.

TAYLOR, R.B. and BASTEN, A. (1976). Suppressor cells in humoral immunity and tolerance. British Medical Bulletin, 32, 152-157.

THOMPSON, D.A., FRAZER, J. and GILMOUR, N.J.L. (1977). Serotypes of Pasteurella haemolytica in ovine pasteurellosis. Research in Veterinary Science, 22, 130-131.

THOMSON, R.G., BENSON, M.L. and SAVAN, M. (1969). Pneumonic pasteurellosis of cattle: microbiology and immunology. Canadian Journal of Comparative Medicine, 33, 194-206.

THRUSFIELD, M.V., SYNGE, B.A. and SCOTT, G.R. (1978). Attempts to cultivate Ehrlichia phagocytophila in vitro. Veterinary Microbiology, 2, 257-266.

TUOMI, J. (1966). Studies in epidemiology of bovine tick-borne fever in Finland and a clinical description of field cases. Annales Mediciniae Experimentalis et Biologiae Fenniae, 44, Supplement 6, 1-62.

TUOMI, J. and VON BONSDROFF, C.H. (1966). Electron microscopy of tick-borne fever agent in bovine and ovine phagocytizing leukocytes. Journal of Bacteriology, 92, 1478-1492.

TUOMI, J. (1967). Experimental studies on bovine tick-borne fever. I. Clinical and haematological data, some properties of the causative agent, and homologous immunity. Acta Pathologica et Microbiologica Scandinavica, 70, 429-445.

TURK, J.L. and WATERS, M.F.R. (1969). Cell-mediated immunity in patients with leprosy. Lancet, 2, 243-246.

UHR, J.W. and FINKELSTEIN, M.S. (1967). The kinetics of antibody formation. Progress in Allergy, 10, 37-43.

UNANUE, E.R., GREY, H.M., RABELLINO, E., CAMPBELL, P. and SCHMIDTHE, J. (1971). Immunoglobulins on the surface of lymphocytes. II. The bone marrow as the main source of lymphocytes with detectable surface-bound immunoglobulin. Journal of Experimental Medicine, 133, 1187-1198.

URQUHART, G.M., MURRAY, M., MURRAY, P.K., JENNINGS, F.W. and BATE, F. (1973). Immunosuppression in Trypanosoma brucei infections in rats and mice. Transactions of the Royal Society of Tropical Medicine and Hygiene, 67, 528-535.

VAN FURTH, R., VAN ZWET, T.L. and LEIJH, P.C.J. (1978). In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes. Handbook of Experimental Immunology, Vol. 2. (Weir, D.M. ed.) 3rd edition pp. 32.1-32.19. Blackwell Scientific Publications, Oxford.

VENN, J.A.J. and WOODFORD, M.H. (1956). An outbreak of tick-borne fever in bovines. Veterinary Record, 68, 132-133.

WARREN, K.S., MAHMOND, A., BOROS, D., RALL, T., MANDEL, M. and CARPENTER, C. (1974). In vivo suppression by cholera toxin of cell-mediated and foreign body inflammatory response. Journal of Immunology, 112, 996-1007.

WATSON, W.A. (1964). Infertility in the ram associated with tick-borne fever infection. Veterinary Record, 76, 1131-1136.

WEDDERBURN, N. and SALAMAN, M.H. (1968). The immunosuppressive effect of Friend virus. II. Reduction of splenic haemolysin-producing cells in primary and secondary responses. Immunology, 15, 439-454.

WHITELAW, D.D., SCOTT, J.M., REID, H.W., HOLMES, P.H., JENNINGS, F.W. and URQUHART, G.M. (1979). Immunosuppression in bovine trypanosomiasis: studies with louping-ill vaccine. Research in Veterinary Science, 26, 102-107.

WILLEMS, F.T.C., MELNICK, J.L. and RAWLS, W.E. (1969). Viral inhibition of the phytohemagglutinin response of human lymphocytes and application to virus hepatitis. Proceedings of the Society for Experimental Biology and Medicine, 130, 652-661.

WILSON, J.C., FOGGIE, A. and CARMICHAEL, M.A. (1964). Tick-borne fever as a cause of abortion and stillbirths in cattle. Veterinary Record, 76, 1081-1084.

WOODS, G.T., SIBINOVIC, K., SEGRE, D. and THURMON, J.C. (1964). Isolation and transmission studies with bovine parainfluenza-3 virus. American Journal of Veterinary Research, 25, 1021-1026.

WOODS, G.T. (1968). The natural history of bovine myxovirus para-influenza-3. Journal of the American Veterinary Medical Association, 152, 771-779.

ZLOTNIK, I., SMITH, C.E.G., GRANT, D.P. and PEACOCK, S. (1970). The effect of immunosuppression on viral encephalitis, with special reference to cyclophosphamide. British Journal of Experimental Pathology, 51, 434-439.

APPENDICES

Appendix Table 1 Daily eosinophil counts of eight TBF-infected sheep: test of symmetry.

Days after inoculation	Mean	Median	Skewness	t
0	249	172	0.658	0.875
1	201(1)	201	0.353	0.445
2	224	145	0.943	1.254
3	145	124	1.079	1.435
4	64	42	1.156	1.537
5	47	38	0.360	0.457
6	31	19	0.453	0.602
7	21	0	0.901	1.195
8	15	0	1.961	2.607**
9	22	0	1.882	2.502*
10	37	15	1.143	1.520
11	63	39	0.936	1.244
12	72	64	0.130	0.173
13	72	64	1.645	2.187*
14	73	52	1.038	1.380
15	75	61	0.993	1.320
16	87	81	0.006	0.008
17	92	70	1.229	1.634
18	119	85	2.543	3.381***
19	88	73	2.418	3.251**
20	208(1)	222	0.221	0.279

(1) mean of seven observations

* $P < 0.050$ i.e. asymmetrical

** $P < 0.010$ i.e. asymmetrical

*** $P < 0.001$ i.e. asymmetrical

Appendix Table 2 Differences between daily eosinophil counts of eight TBF-infected sheep: test of symmetry

Difference	Mean	Median	Skewness	t
0-1	22(1)	-16	1.431	1.803
0-2	26	16	1.450	1.929
0-3	105	74	0.666	0.885
0-4	186	146	0.851	1.131
0-5	203	169	0.665	0.884
0-6	218	160	0.504	0.671
0-7	228	159	0.716	0.952
0-8	234	188	0.600	0.798
0-9	228	166	0.877	1.166
0-10	200	157	1.113	1.480
0-11	187	124	0.348	0.463
0-12	178	120	0.754	1.003
0-13	177	126	0.576	0.766
0-14	177	84	0.861	1.145
0-15	176	88	0.712	0.947
0-16	164	108	0.563	0.748
0-17	158	118	0.820	1.090
0-18	132	112	-0.124	0.166
0-19	162	106	0.823	1.094
0-20	8(1)	-18	0.033	0.042

(1)Mean of seven observations

Appendix Table 3 Daily monocyte counts of eight TBF-infected sheep: test of symmetry

Day	Mean	s.d.	Median	Skewness	t
0	153.25	54.02	164	-0.880	1.170
1	177.75	67.54	178	-0.341	0.453
2	151.00	83.94	124	0.654	0.870
3	168.38	110.05	136	0.841	1.118
4	300.00	233.49	206	1.400	1.862
5	394.88	341.48	240	1.397	1.858
6	279.25	185.41	222	1.722	2.290*
7	233.25	124.98	198	0.575	0.764
8	209.88	137.55	163	0.773	1.028
9	197.00	117.56	164	1.610	2.141*
10	172.12	109.93	167	0.673	0.895
11	127.00(1)	55.03	128	-0.216	0.272
12	144.50	68.22	122	0.030	0.040
13	116.28(1)	46.61	112	0.058	0.073
14	109.00(1)	40.29	104	0.050	0.063
15	132.25	94.81	113	1.550	2.061*
16	161.88	84.58	138	1.573	2.092*
17	194.12	75.95	189	-0.167	0.222
18	162.25	48.30	152	1.393	1.852
19	134.62	70.08	120	0.832	1.106
20	191.50	72.05	202	-0.102	0.136

(1) Mean of seven observations

* $P < 0.050$ i.e. asymmetrical

Appendix Table 4 Differences between daily monocyte counts of eight TBF-infected sheep: test of symmetry

Difference	Mean	s.d.	Median	Skewness	t
0-1	-24	67	-2	-0.698	0.928
0-2	2	121	4	-0.733	0.975
0-3	-15	120	10	-0.366	0.487
0-4	-147	219	-38	-1.238	1.646
0-5	-242	325	-117	-1.270	1.689
0-6	-126	177	-114	-1.028	1.367
0-7	-80	134	-96	0.623	0.828
0-8	-56	131	-96	-0.073	0.097
0-9	-44	153	-14	-1.400	1.862
0-10	-19	146	-35	-0.639	0.850
0-11	40(1)	77	53	-0.900	1.134
0-12	9	108	37	-0.842	1.120
0-13	46(1)	40	57	-1.419	1.788
0-14	53(1)	45	67	-0.896	1.129
0-15	21	94	11	-0.402	0.534
0-16	-104	127	22	1.218	1.620
0-17	-41	109	-44	0.335	0.445
0-18	-9	60	16	-0.688	0.915
0-19	19	52	18	-0.344	0.457
0-20	-38	95	17	-0.238	0.316

(1)Mean of seven observations

Appendix Table 5 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in eight TBF-infected sheep

Days after inoculation	Sheep No. 291	292	294	295	297	299	319	320
1	13.8	5.2	-	12.1	20.3	30.9	23.6	28.2
2	-11.9	0.04	-13.4	-26.4	-3.8	31.4	13.8	29.3
3	-51.0	-25.0	-30.0	-61.0	-30.9	-15.6	-23.9	-21.4
4	-60.5	-46.7	-55.1	-80.0	-55.8	-56.3	-55.5	-36.7
5	-70.0	-68.5	-50.9	-84.9	-66.5	-62.9	-60.7	-53.3
6	-76.9	-69.4	-65.1	-79.0	-62.0	-69.4	-65.9	-71.4
7	-66.6	-62.8	-39.2	-77.0	-61.6	-74.9	-65.2	-53.0
8	-72.3	-61.6	-17.3	-64.0	-62.8	-38.4	-52.0	-59.8
9	-58.9	-53.5	-18.1	-47.0	-57.0	-39.4	-24.1	-63.3
10	-48.6	-38.9	-18.9	-29.8	-52.0	-18.0	-44.6	-51.0
11	-43.4	-39.9	-25.5	-28.0	-17.6	4.8	-	-27.5
12	-38.2	-41.0	-16.3	-32.8	-14.0	14.8	-20.2	-30.4
13	-49.6	-20.5	-6.6	-40.0	-39.6	-	-24.6	-32.5
14	-41.0	-37.2	-14.3	-48.8	-35.0	-	-58.9	-55.6
15	-54.0	-27.9	2.8	-22.7	-7.9	5.8	-46.5	-27.1
16	-46.5	-9.3	9.4	-15.0	-11.7	8.7	-34.0	-39.8
17	-42.6	-9.8	15.9	-7.7	15.5	8.5	-29.3	-24.1
18	-36.7	-4.9	2.1	-3.6	10.9	11.4	14.2	-16.8

Appendix Table 6 Daily eosinophil counts expressed as percentages of the pre-inoculation value in eight TBF-infected sheep

Days after inoculation	Sheep No. 291	292	294	295	297	299	319	320
1	13.5	5.2	-	33.0	15.9	15.1	-29.0	-26.0
2	18.6	50.0	20.3	-33.0	14.5	-40.0	-3.0	-22.0
3	54.8	-10.9	-12.7	43.8	-100.0	-49.0	-61.5	-36.7
4	-100.0	-21.0	-57.0	-34.0	-100.0	-75.0	-60.9	-32.0
5	-100.0	-32.0	-80.8	-100.0	-100.0	-100.0	-75.0	-74.0
6	-100.0	-70.6	-91.0	-100.0	-46.0	-100.0	-100.0	-84.0
7	-100.0	-69.6	-90.6	-100.0	-54.0	-100.0	-100.0	-100.0
8	-100.0	-100.0	-90.0	-100.0	-47.0	-100.0	-100.0	-100.0
9	-100.0	-100.0	-76.0	-100.0	-41.0	-75.6	-100.0	-100.0
10	-100.0	-100.0	-71.9	-58.6	-40.0	-42.7	-100.0	-100.0
11	47.4	-100.0	-100.0	-57.0	-34.0	-30.0	-	-100.0
12	-49.0	-100.0	-72.0	-55.0	-32.0	-21.6	-78.0	-72.0
13	-9.8	-70.6	-67.8	-63.0	-26.0	-	-79.8	-74.0
14	-9.0	-28.0	-57.8	-62.0	-23.8	-	-76.9	-70.0
15	-12.7	-21.0	-46.5	-52.8	-4.0	-25.9	-47.0	-47.0
16	7.2	-17.0	-40.0	10.0	-17.0	-25.0	-41.0	-37.0
17	-17.0	-12.8	-21.8	21.6	-5.8	-22.7	-30.0	-32.0
18	-16.0	-18.6	-22.0	25.6	53.0	-20.0	-35.8	-32.0

Appendix Table 7 Daily neutrophil counts expressed as percentages of the pre-inoculation value in eight TBF-infected sheep

Days after inoculation	Sheep No. 291	292	294	295	297	299	319	320
1	13.8	7.4	-	-35.0	5.2	14.0	20.0	13.9
2	24.1	4.9	2.5	-42.3	30.0	10.1	7.6	22.3
3	23.5	10.0	14.0	-19.5	53.6	23.5	52.0	27.9
4	4.0	9.3	-5.2	-53.9	5.4	22.9	15.9	47.0
5	6.0	8.5	-28.3	-40.1	10.0	21.4	36.2	23.5
6	7.6	-4.8	-19.4	-47.2	-26.7	0.03	56.6	2.5
7	-33.5	-5.7	-44.8	-44.2	-43.4	-72.2	23.6	3.7
8	-54.7	-28.6	-72.3	-61.9	-73.0	-51.3	24.6	11.4
9	-83.4	-3.8	-70.9	-74.8	-78.7	-78.7	-21.6	-57.4
10	-81.8	-71.4	-69.5	-87.7	-84.3	-88.2	-82.6	-78.1
11	-80.9	-75.9	-63.5	-89.6	-69.3	-85.6	-	-70.0
12	-80.2	-80.4	-62.8	-77.8	-70.7	-81.8	-60.3	-63.2
13	-73.8	-70.0	-65.5	-88.3	-85.9	-	-59.4	-56.1
14	-70.5	-17.7	-35.3	-79.7	-73.4	-	-33.7	-78.4
15	-59.4	-67.2	-39.0	-89.5	-76.1	-71.4	-85.7	-86.2
16	-41.5	-66.2	-36.0	-80.5	-61.6	-80.8	-77.8	-72.4
17	-16.8	-49.3	-32.9	-71.5	-47.1	-78.7	-71.8	-70.0
18	-34.8	-35.3	-30.5	-31.0	-40.3	-71.6	-9.9	-62.8

Appendix Table 8 Daily monocyte counts expressed as percentages of the pre-inoculation value in eight TBF-infected sheep

Days after inoculation	Sheep No. 291	292	294	295	297	299	319	320
1	13.9	4.9	-	-25.2	43.9	57.7	6.4	-26.3
2	-59.3	0.5	-6.1	-66.6	50.4	17.1	79.0	-58.3
3	32.2	-10.9	-56.8	-40.8	56.9	2.1	70.4	-68.9
4	56.4	15.1	22.3	-1.7	74.2	60.6	37.6	-25.3
5	80.7	50.9	30.1	23.6	80.3	59.2	55.7	-45.4
6	69.9	15.4	45.7	-10.3	62.7	57.8	64.6	-46.8
7	56.0	-7.8	29.4	45.9	13.0	68.1	69.1	-78.9
8	53.3	-19.3	13.6	-63.7	-47.0	65.4	66.2	44.7
9	20.6	-33.5	7.0	-60.9	-24.5	79.6	73.0	-37.8
10	24.7	-53.4	20.6	-78.7	-1.9	75.1	71.5	-64.1
11	-4.9	-32.9	-12.3	-78.7	-34.6	52.5	-	-48.3
12	3.3	-36.6	-16.4	-77.5	-32.6	57.8	75.0	-47.3
13	-9.8	-41.3	-63.0	-43.6	-5.8	-	18.3	-27.2
14	-49.0	-28.2	-63.0	-5.1	-47.0	-	28.3	-32.0
15	-56.0	42.9	-59.5	-5.1	7.2	32.8	-22.4	-68.8
16	-46.0	-67.0	-10.9	-16.6	28.4	73.3	67.7	-9.5
17	-68.6	-10.3	30.8	-4.0	49.6	67.0	64.8	-28.2
18	-53.3	-16.7	-17.1	-32.7	41.6	36.9	54.6	-19.1

Appendix Table 9 Serum antibody titres (log_2 reciprocal) to *Pasteurella haemolytica* of newly bought sheep inoculated with TBF-infected blood

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	A_{11}	A_{12}
184	A.	3	3	2	2	2	2	2	3	0	2	0	0
	B.	0	0	0	2	0	2	0	3	0	0	0	0
	C.	0	0	0	0	0	2	0	2	0	0	0	0
	D.	0	0	0	0	0	4	0	4	2	0	0	2
186	A.	2	2	0	0	0	0	0	0	0	0	0	0
	B.	2	0	0	0	0	0	0	0	0	0	0	0
	C.	3	0	0	0	0	0	0	0	0	0	0	0
	D.	4	0	2	0	0	2	0	2	0	0	0	0
188	A.	0	0	0	0	3	2	0	2	2	0	0	2
	B.	0	0	0	0	3	0	0	2	2	0	0	3
	C.	0	0	0	0	2	0	0	0	0	0	0	0
	D.	3	0	0	0	2	0	0	0	0	0	0	0
189	A.	1	0	0	0	0	2	2	0	2	0	0	2
	B.	1	0	0	0	0	0	2	0	2	0	0	2
	C.	3	0	0	0	0	0	2	0	0	0	0	0
	D.	5	0	2	3	0	0	2	0	0	2	0	0
191	A.	1	2	0	3	0	3	0	4	0	2	0	0
	B.	2	2	0	2	0	2	0	2	0	2	0	0
	C.	4	2	0	2	0	2	0	2	0	0	0	0
	D.	5	3	0	0	0	3	0	2	0	0	0	0
203	A.	0	0	0	0	0	3	0	3	0	3	0	0
	B.	0	0	0	0	0	3	0	3	0	2	2	0
	C.	0	0	0	2	0	3	0	4	2	2	5	0
	D.	0	0	0	2	0	2	2	3	2	2	5	0

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 9 (continued) Serum antibody titres (log_2 reciprocal) to Pasteurella haemolytica of newly bought sheep inoculated with TBF-infected blood

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	A_{11}	A_{12}
204	A.	0	2	2	3	2	2	0	3	0	0	0	2
	B.	0	2	2	3	2	2	0	2	0	5	3	2
	C.	0	0	0	3	0	0	0	2	0	6	4	0
	D.	2	0	0	3	0	0	2	3	2	4	4	0
222	A.	0	1	2	1	0	0	0	0	0	1	1	1
	B.	0	2	2	2	0	4	1	1	0	2	3	0
	C.	0	1	2	2	0	4	2	1	0	1	5	0
	D.	0	1	3	2	0	5	2	2	1	1	5	0
223	A.	0	2	2	2	0	0	0	0	0	2	0	0
	B.	0	1	2	1	0	2	0	1	0	3	3	0
	C.	0	2	3	1	0	4	0	2	0	3	4	0
	D.	0	2	3	2	0	4	0	1	0	3	4	0
101	A.	4	3	3	4	0	0	4	0	3	3	4	3
	B.	4	4	4	4	0	0	5	0	5	3	4	4
	C.	4	3	3	3	0	0	3	0	6	3	3	3
	D.	2	2	3	3	0	0	3	0	5	3	3	3
103	A.	3	3	0	0	0	2	4	0	3	0	4	4
	B.	4	3	0	0	0	0	5	0	6	0	4	3
	C.	4	4	1	1	0	0	4	0	7	2	5	2
	D.	4	4	1	1	0	0	4	0	6	?	5	3

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 10 Serum antibody titres (log_2 reciprocal) to Pasteurella haemolytica of the newly bought control sheep

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	A_{11}	A_{12}
183	A.	0	0	0	2	3	0	0	2	0	2	0	0
	B.	0	0	0	2	0	0	0	2	0	0	0	0
	C.	0	0	0	2	0	0	0	2	0	0	0	0
	D.	0	2	2	2	0	3	0	2	0	0	3	0
185	A.	0	0	0	3	0	2	0	2	0	0	2	0
	B.	0	0	0	0	0	2	0	2	0	0	0	0
	C.	0	0	0	0	0	2	0	2	0	0	0	0
	D.	3	0	0	0	0	3	0	2	0	0	3	0
224	A.	0	0	1	1	0	0	0	1	0	1	0	0
	B.	1	1	2	1	0	0	0	1	0	2	0	0
	C.	1	1	2	1	0	0	0	1	0	1	0	0
	D.	1	1	1	1	0	0	0	2	0	0	0	0
225	A.	0	0	1	1	0	1	0	0	0	1	1	0
	B.	0	0	1	1	0	3	1	0	0	1	1	0
	C.	0	0	2	1	0	5	2	0	0	2	0	0
	D.	0	0	2	1	0	4	1	0	0	2	0	2
226	A.	0	0	1	1	0	0	0	0	0	0	0	0
	B.	0	0	2	1	0	0	0	0	0	0	0	0
	C.	0	0	1	2	0	2	1	1	0	0	0	0
	D.	0	0	1	2	0	2	2	2	0	0	0	0
227	A.	0	0	2	2	0	0	1	0	1	1	2	2
	B.	0	0	1	1	0	1	1	0	1	2	5	3
	C.	1	0	1	1	1	4	2	0	0	2	6	3
	D.	1	1	1	1	1	3	1	0	0	1	4	3
100	A.	0	4	2	0	5	0	5	4	4	2	2	3
	B.	0	5	0	0	3	0	3	4	4	0	5	3
	C.	0	5	0	0	4	0	4	4	4	0	4	4
	D.	0	5	0	0	3	0	3	3	3	0	4	2

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 10 (continued) Serum antibody titres (log_2 reciprocal) to Pasteurella haemolytica of the newly bought control sheep

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	A_{11}	A_{12}
102	A.	3	3	3	4	4	0	0	4	3	3	4	0
	B.	0	2	4	4	3	0	0	4	4	3	4	3
	C.	0	3	4	3	3	0	0	3	3	2	3	3
	D.	0	2	3	3	3	0	0	4	3	2	4	3
193	A.	3	0	0	0	3	3	3	4	0	1	4	0
	B.	2	0	0	0	3	3	2	3	0	2	3	0
	C.	4	0	0	2	3	3	2	3	0	2	2	0
	D.	0	3	3	2	3	2	2	3	0	2	3	0
194	A.	0	2	4	2	2	3	0	3	0	2	2	0
	B.	0	3	3	3	3	3	0	3	0	2	2	0
	C.	0	3	3	2	2	3	0	3	0	2	2	0
	D.	0	3	3	3	3	3	0	3	0	3	3	0
182	A.	0	2	0	2	2	3	2	3	0	3	3	0
	B.	0	0	0	0	0	2	0	2	0	0	0	0
	C.	0	0	0	0	0	2	0	2	0	0	0	0
	D.	0	0	0	0	0	2	0	3	0	0	0	0
201	A.	0	0	2	2	0	3	0	2	2	2	0	0
	B.	0	0	2	2	0	3	0	2	2	2	3	0
	C.	0	0	0	0	0	2	0	2	0	0	3	0
	D.	2	3	0	3	2	3	0	3	0	0	3	0
202	A.	2	2	2	3	2	2	0	2	0	0	0	0
	B.	0	0	0	2	0	0	0	2	0	0	2	0
	C.	0	0	0	0	0	0	2	3	0	2	2	0
	D.	0	0	0	0	0	0	2	3	2	2	2	2
208	A.	0	0	0	0	2	0	0	2	2	2	0	2
	B.	0	0	0	0	0	0	0	2	0	0	2	2
	C.	0	2	2	0	0	2	2	2	0	0	2	2
	D.	2	3	3	2	0	2	2	2	0	0	2	0

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 11 Serum antibody titres (log_2 reciprocal) to Pasteurella haemolytica of acclimatised sheep inoculated with TBF-infected blood

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	A_{11}	A_{12}
205	A.	1	3	3	3	0	0	2	3	0	2	3	0
	B.	1	0	2	0	0	0	1	1	0	1	1	0
	C.	0	0	0	0	0	0	0	1	0	1	1	1
	D.	0	0	0	0	0	0	0	1	0	1	1	1
206	A.	0	2	3	3	2	0	0	3	0	1	2	0
	B.	0	1	1	2	0	0	0	1	0	3	1	0
	C.	0	0	2	2	0	0	0	1	0	4	2	0
	D	0	0	2	1	0	0	0	1	0	5	1	0
207	A.	0	0	3	2	0	0	0	2	0	0	2	0
	B.	0	0	0	0	0	0	0	1	0	4	1	0
	C.	0	0	0	0	0	0	0	0	2	5	3	0
	D.	0	0	0	0	0	0	0	0	2	5	4	0
190	A	2	3	0	2	2	3	2	3	0	4	1	0
	B.	1	3	0	3	2	3	3	4	0	3	3	0
	C.	1	3	0	2	2	3	4	4	2	2	5	0
	D.	1	0	2	2	2	2	2	4	2	3	4	2
192	A.	0	3	0	0	3	3	0	3	0	3	1	0
	B.	0	3	0	0	2	3	0	3	0	3	2	0
	C.	0	3	0	0	2	2	0	2	0	3	4	0
	D.	2	2	2	2	2	2	2	3	0	3	4	4
187	A.	0	0	0	2	2	0	0	2	0	0	0	0
	B.	0	3	0	0	0	0	0	2	0	0	2	0
	C.	0	3	2	0	0	2	0	4	0	2	2	0
	D.	0	3	2	0	0	2	0	2	0	2	2	0

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 12 Serum antibody titres (log_2 reciprocal) to *Pasteurella haemolytica* of acclimatised control sheep

Sheep No.		Serotype A_1	A_2	T_3	T_4	A_5	A_6	A_7	A_8	A_9	T_{10}	T_{11}	T_{12}
180	A.	0	1	0	0	0	1	0	3	0	0	0	0
	B.	0	0	0	0	0	3	0	2	0	0	0	0
	C.	0	0	0	0	0	4	0	2	0	0	0	0
	D.	0	0	2	1	0	4	0	2	0	0	2	0
181	A.	0	1	0	0	0	3	2	3	0	0	3	0
	B.	0	0	0	2	0	2	2	2	0	0	2	0
	C.	2	0	2	4	2	2	2	2	0	0	2	2
	D.	2	0	2	6	0	2	0	2	0	2	2	2
195	A.	1	1	1	2	2	0	0	1	0	0	0	0
	B.	0	0	4	1	2	0	0	0	0	0	0	0
	C.	0	0	5	1	1	0	0	0	0	1	0	0
	D.	0	0	3	1	1	0	0	0	0	2	0	0
220	A.	1	0	1	0	0	2	0	0	0	0	0	0
	B.	1	0	2	0	0	2	0	0	0	0	0	0
	C.	1	0	2	2	2	2	0	2	2	3	2	0
	D.	2	0	2	2	2	2	0	2	3	3	3	2
221	A.	0	0	3	0	2	0	0	3	0	0	0	0
	B.	0	0	2	0	1	0	0	2	0	0	0	0
	C.	0	2	3	0	2	2	0	2	0	3	0	0
	D.	0	2	2	2	2	0	0	2	0	2	0	0
228	A.	1	2	0	2	3	0	2	2	3	0	3	0
	B.	0	2	2	2	2	0	2	2	2	0	3	0
	C.	0	2	3	2	2	0	2	2	3	0	2	0
	D.	0	2	3	0	0	0	2	2	2	0	2	0
229	A.	1	0	0	1	0	0	2	2	4	3	3	3
	B.	0	0	0	2	0	0	3	0	2	2	2	3
	C.	0	2	2	3	0	0	0	0	0	0	2	2
	D.	0	2	1	0	0	0	0	0	0	0	2	2

A. Immediately before inoculation
B. Ten days after inoculation
C. 20 days after inoculation
D. 30 days after inoculation

Appendix Table 13 Anovars of the antibody titres of sheep shedding P. haemolytica.

Days after inoculation	Source of difference	Degrees of freedom	Mean square	Variance ratio
0	Between TBF-infected and non-infected sheep	1	0.2077	0.46
	Between newly bought and acclimatised sheep	1	0.8077	1.77
	Interaction	1	3.1923	7.001*
	Error	9	0.4556	
10	Between TBF-infected and non-infected sheep	1	0.9256	0.98
	Between newly bought and acclimatised sheep	1	1.1447	1.21
	Interaction	1	3.1220	3.30
	Error	9	0.9444	
20	Between TBF-infected and non-infected sheep	1	0.7410	2.38
	Between newly bought and acclimatised sheep	1	0.8077	2.60
	Interaction	1	1.9590	6.30*
	Error	9	0.3111	
30	Between TBF-infected and non-infected sheep	1	0.2077	0.26
	Between newly bought and acclimatised sheep	1	0.8077	1.01
	Interaction	1	0.0923	0.12
	Error	9	0.8000	

* $P < 0.050$

Appendix Table 14 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with PI-3 virus alone

Days after inoculation	Sheep No. 991	996	61	62	87	88	93	94	107	108
1	17.8	3.0	2.4	15.0	0.3	3.8	33.2	0.1	3.5	11.0
2	17.7	-26.0	-8.0	5.3	-6.0	13.5	4.0	8.2	-7.0	18.2
3	8.2	-16.2	-19.0	-4.0	-6.4	16.6	7.0	1.3	-14.0	17.5
4	7.7	-6.0	-7.0	-2.0	25.0	7.8	3.3	12.0	-5.2	-6.5
5	-3.0	-9.0	-4.0	-11.4	24.0	9.2	7.9	-2.0	-2.0	-7.6
6	-3.2	-6.0	-16.0	-0.9	20.6	6.8	0.2	-5.0	-3.0	-6.0
7	-2.0	-15.0	-18.4	-4.0	23.6	7.8	13.0	-7.0	-6.0	10.0
8	9.5	-5.0	-41.1	-23.0	17.6	-18.0	-17.0	-12.0	-4.0	19.6
9	8.7	4.3	-18.0	-9.0	18.1	-12.0	-21.0	-33.0	4.5	19.3
10	9.5	5.0	-66.0	-25.0	19.7	-12.0	-13.0	-15.0	9.6	15.3
11	7.0	7.3	-32.0	-8.0	25.7	-3.0	-11.0	-15.0	7.5	6.0
12	1.7	2.3	-13.6	3.4	31.0	-17.0	-6.2	1.6	4.7	15.0
13	7.8	1.2	-12.3	6.2	24.5	2.1	4.8	18.4	8.4	10.3
14	14.7	9.2	17.0	5.8	21.2	8.0	9.0	22.4	8.2	14.0

Appendix Table 15 Daily neutrophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with PI-3 virus alone

Days after inoculation	Sheep No. 991	996	61	62	87	88	93	94	107	108
1	11.6	28.3	4.0	0.2	19.8	43.6	19.2	0.1	0.5	4.0
2	3.8	35.0	3.8	-35.0	0.4	32.8	30.4	2.2	4.3	2.0
3	6.8	10.5	1.5	10.5	-19.0	23.6	11.0	-15.0	1.0	-18.0
4	2.0	-12.0	-1.7	10.4	-14.0	16.0	40.0	-5.0	9.0	-6.0
5	-13.2	-2.0	-18.0	6.8	-2.0	17.5	34.4	-4.4	3.6	-22.0
6	-6.3	-1.0	8.6	9.2	13.3	16.7	25.4	-20.3	-14.2	-1.0
7	-2.1	17.4	3.8	2.5	16.8	6.0	3.0	-22.0	-23.0	-21.7
8	-7.3	16.6	32.5	27.6	26.3	-10.0	8.5	-28.2	-9.2	-7.4
9	-34.2	14.0	19.0	14.8	29.0	-28.0	0.6	-31.0	-21.0	-31.2
10	-7.3	16.6	17.0	31.7	25.8	-22.0	-13.0	-0.5	-21.0	-28
11	34.2	20.3	12.5	16.2	23.0	-30.3	-7.0	-39.0	-21.0	-25
12	29.2	2.8	9.3	9.2	18.6	-20.3	19.7	-13.3	-8.0	-21
13	19.7	12.5	1.2	10.0	13.2	-37.0	17.5	6.0	4.2	10
14	6.8	24.0	1.2	0.2	1.8	15.0	5.4	20.6	9.5	15.7

Appendix Table 16 Daily eosinophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with PI-3 virus alone

Days after inoculation	Sheep No. 991	996	61	62	87	88	93	94	107	108
1	9.6	-29.0	-39.5	-25.7	38.4	36.0	38.8	1.0	16.0	-48.2
2	8.0	-51.4	-40.8	-21.3	33.4	54.3	-19.0	1.9	8.0	-46.5
3	6.8	-11.7	-44.3	-38.0	51.0	21.0	-29.5	51.0	5.0	-8.5
4	-20.8	-21.3	-31.7	-37.5	16.2	42.2	-27.8	7.3	21.0	-50.5
5	-42.8	-21.3	-33.0	-59.5	19.5	-23.3	-77.8	50.0	-84.6	-0.5
6	-60.8	-38.4	-32.6	-67.0	38.0	-44.0	-79.7	-1.0	-1.0	37.0
7	-0.1	-50.6	-34.0	-58.4	48.2	-45.0	-79.5	-10.8	-27.0	14.0
8	-13.5	-58.4	-50.4	-58.0	49.2	-12.6	-65.6	44.5	-54.5	43.0
9	-29.4	-40.6	-50.0	-1.7	-35.7	-16.6	-24.2	77.0	-36.7	63.4
10	-13.5	-48.4	-38.0	-27.5	-35.0	-26.6	-30.4	79.2	-34.0	52.0
11	-19.4	-35.5	-32.0	-26.6	-32.7	-14.4	-28.8	76.6	13.5	76.0
12	-13.5	-11.3	-0.4	-26.0	-31.5	-14.8	-41.2	67.8	13.0	36.5
13	9.1	-13.6	5.8	16.3	-35.7	-14.4	-14.6	17.8	5.0	48.8
14	10.8	14.0	21.3	21.8	1.4	5.0	26.3	2.0	1.4	53.2

Appendix Table 17 Daily monocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with PI-3 virus alone

Days after inoculation	Sheep No. 991	996	61	62	87	88	93	94	107	108
1	7.9	7.2	0.8	1.7	8.4	12.0	2.0	1.0	1.0	1.0
2	10.2	4.2	5.8	2.4	10.2	1.3	3.4	9.0	6.3	3.3
3	6.9	13.4	15.3	-4.3	-6.0	1.8	2.0	-5.7	-9.5	-8.5
4	-25.6	-16.0	3.4	-2.6	-14.3	-13.4	8.0	-6.8	-4.7	-4.0
5	-28.5	-36.0	-17.0	-8.3	19.5	-13.3	-25.5	-0.5	-7.0	-10.0
6	-15.6	-49.2	-26.0	-0.8	19.3	-15.8	-19.3	27.6	-1.0	-29.4
7	-13.0	-9.8	-7.8	-29.6	21.4	9.0	-18.6	24.8	-12.2	14.3
8	-9.2	-2.9	16.5	-25.0	19.0	12.6	-13.8	6.4	-9.0	23.0
9	8.4	-0.4	0.4	-10.8	21.8	14.4	15.7	7.5	-4.7	10.0
10	9.2	-5.4	4.5	-1.3	8.6	13.2	42.6	16.4	-1.0	4.4
11	8.9	3.2	11.8	0.4	8.6	27.3	25.3	13.3	-0.5	5.8
12	8.9	11.5	0.4	12.0	5.6	6.4	4.8	15.5	2.5	5.5
13	7.1	3.7	1.3	1.3	2.6	8.7	6.4	8.4	5.2	3.3
14	4.6	8.0	1.7	2.5	7.8	14.4	2.4	3.6	7.2	7.2

Appendix Table 18 Daily lymphocyte counts expressed as the percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Days after inoculation	Sheep No. 133	134	149	150	151	988	994	63	89	90
1	0.6	1.5	15.0	1.0	0.6	0.4	6.7	4.4	1.0	0.8
2	0.3	0.03	27.3	-4.5	0.2	-15.0	-4.1	11.5	-24.2	-6.4
3	11.2	4.6	-23.5	-39.6	-44.6	-45.6	-22.6	-54.0	-24.6	-13.4
4	-29.0	-8.7	-72.0	-77.6	-63.4	-61.0	-31.8	-49.2	-27.3	-39.7
5	-38.0	-46.7	-54.7	-66.7	-67.6	-32.5	-53.4	-46.6	-30.5	-49.5
6	-70.3	-55.7	-51.5	-62.0	-62.0	-48.3	-65.0	-59.0	-45.9	-57.9
7	-58.6	-57.0	-61.7	-63.0	-63.4	-66.1	-53.4	-63.7	-49.3	-65.0
8	-56.0	-52.0	-43.0	-59.2	-63.0	-51.3	-50.0	-46.8	-50.6	-46.5
9	-61.8	-50.0	-48.0	-58.0	-61.3	-56.2	-45.0	-52.5	-50.6	-41.3
10	-45.5	-52.0	-31.5	-42.6	-60.4	-46.7	-38.0	-32.5	-48.0	-33.8
11	-44.0	-30.7	-26.3	-37.8	-49.3	-48.0	-40.3	-16.5	-39.4	-40.5
12	-47.0	-43.0	-25.7	-16.1	-45.6	-59.3	-32.4	-16.6	-14.3	-43.6
13	-51.5	-40.6	-25.2	-16.4	-49.1	-44.5	-30.8	-22.2	-35.0	-60.8
14	-60.5	-59.6	-24.6	-12.7	-51.4	-29.5	-32.7	-23.4	-26.9	-48.0
15	-49.0	-40.4	-17.7	8.2	-49.6	-38.5	-32.4	-26.2	-30.0	-40.3
16	-46.8	-45.0	-13.5	6.1	-37.0	-44.5	-30.2	-6.3	-24.2	-35.0
17	-43.2	-43.4	-6.4	15.2	-37.0	-45.6	-21.3	-13.4	-23.6	-42.8
18	-47.3	-45.8	-1.4	8.1	-44.0	-46.6	-22.0	-16.2	-1.2	-26.3

Appendix Table 19 Daily neutrophil counts expressed as percentages of the pre-inoculation values in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Days after inoculation	Sheep No. 133	134	149	150	151	988	994	63	89	90
1	0.6	15.0	3.0	40.7	40.8	0.4	2.8	7.0	9.7	17.0
2	0.3	12.6	19.2	45.2	9.6	10.5	14.2	4.3	36.0	16.4
3	14.6	16.4	31.7	53.3	60.0	30.6	11.6	47.3	34.0	25.8
4	15.0	38.4	-16.0	25.9	33.5	5.2	-14.5	-1.0	34.3	1.0
5	44.3	29.6	-11.0	-4.0	37.3	48.0	-5.8	-27.5	38.0	6.4
6	30.2	34.3	-1.0	-41.5	33.5	7.6	-42.5	-34.3	33.6	10.0
7	-30.8	4.4	-11.2	-37.5	20.0	-53.0	-35.2	-32.5	33.5	-37.5
8	-19.6	18.5	-5.8	-18.4	13.7	-69.8	-41.5	-36.4	5.0	-45.0
9	-6.4	25.4	-35.8	-78.3	6.0	-77.6	-42.0	-40.8	3.8	-42.8
10	-75.0	5.7	-66.7	-75.0	-0.5	-75.2	-66.0	-67.4	0.4	-55.8
11	-81.0	10.5	-81.3	-66.0	-26.5	-60.8	-58.8	-63.0	-47.6	-38.7
12	-78.7	-36.0	-81.4	-79.5	-69.8	-67.0	-57.6	-66.5	-42.7	-44.6
13	-80.3	-14.8	-73.5	-77.0	-57.0	-51.0	-63.5	-61.4	-43.6	-43.3
14	-77.2	-83.4	-77.0	-58.2	-80.0	-52.0	-55.8	-64.4	-55.3	-40.2
15	-78.6	-71.0	-78.7	-34.2	-66.5	-41.0	-40.5	-28.6	-45.8	-48.4
16	-72.0	-67.6	-66.8	-34.0	-72.5	-39.3	-20.7	-20.0	-37.4	-55.5
17	-61.0	-63.8	-52.6	-41.5	-54.0	-39.4	-38.2	-11.0	-19.2	-39.0
18	-72.3	-71.6	-61.0	3.0	-26.5	-42.4	-22.0	16.4	6.7	-15.7

Appendix Table 20 Daily eosinophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Days after inoculation	Sheep No. 133	134	149	150	151	988	994	63	89	90
1	2.8	-0.8	17.0	36.3	49.0	1.0	6.5	17.0	1.0	3.8
2	42.8	-0.8	5.4	-33.0	-14.4	45.0	-53.4	15.5	53.0	2.7
3	-35.7	-9.7	-79.0	-54.4	-72.4	32.0	-56.0	-3.4	-52.5	-49.0
4	-38.7	-100	-100	-85.0	-84.3	26.0	-100	-70.6	-52.0	-100
5	-85.5	-100	-100	-90.0	-77.7	2.0	-100	-71.7	-100	-100
6	-87.5	-100	-83.0	-95.0	-84.3	-27.0	-100	-100	-100	-100
7	-76.0	-100	-82.0	-89.0	-80.0	-100	-100	-100	-21.7	-64.4
8	-81.5	-100	-84.7	-95.0	-84.7	-100	-100	-100	-7.1	-41.0
9	-100	-100	-83.0	-79.0	-87.7	-100	-100	-100	-54.4	-56.4
10	-72.7	-100	-84.0	-76.0	-84.2	-100	-14.5	-69.0	-17.0	-21.0
11	-83.2	-16.0	-84.0	-74.0	-100	-100	-14.5	-65.2	-21.0	-13.2
12	-83.0	-42.0	-88.0	-93.6	-100	-60.0	-6.3	20.5	-30.7	-38.0
13	-70.7	-20.0	-78.0	-100	-93.5	-40.0	-58.6	23.5	-13.7	-35.4
14	30.3	-30.0	34.0	-100	-82.4	-47.0	-21.5	25.0	-11.4	-9.6
15	32.4	-4.4	27.0	-100	41.0	-38.0	25.8	20.0	-3.4	-21.3
16	33.5	33.7	17.0	-74.3	28.0	40.5	21.8	17.0	-12.6	-15.0
17	21.7	30.3	14.0	8.0	21.0	22.6	23.2	28.0	-23.0	-35.0
18	20.8	31.0	16.0	5.0	12.4	16.4	22.0	25.0	3.0	-25.0

Appendix Table 21 Daily monocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-1} and PI-3 virus

Days after inoculation	Sheep No. 133	134	149	150	151	988	994	63	89	90
1	-5.5	-60.2	-73.3	-29.2	-1.5	-1.0	6.5	-0.9	-0.5	-1.6
2	-6.4	-20.4	-30.6	6.0	-4.7	4.0	28.3	-0.8	-13.7	-8.4
3	11.8	-11.9	-14.2	15.6	-14.6	19.0	24.5	18.3	28.6	-54.8
4	23.9	-37.4	-1.6	15.5	-31.7	43.1	30.9	23.9	47.6	-2.2
5	38.8	-46.5	-14.7	31.1	-14.2	51.6	28.9	4.1	45.8	32.6
6	30.9	-20.7	-25.2	40.7	-8.3	56.5	62.8	49.7	29.3	31.9
7	36.6	-10.3	-12.3	43.1	47.5	64.1	67.4	49.2	36.2	43.6
8	29.8	-9.9	-20.6	41.4	52.7	62.8	64.4	40.9	50.3	55.9
9	47.9	-8.8	-16.6	41.1	35.0	48.3	1.1	42.5	51.9	56.4
10	40.4	-18.6	-6.1	20.0	51.0	38.0	10.4	59.4	35.6	50.9
11	43.7	-53.8	-2.1	46.2	36.5	36.9	12.6	46.9	51.5	28.6
12	2.7	-10.8	-0.5	45.1	38.5	23.9	-37.7	57.2	51.1	12.3
13	6.8	-14.3	-4.0	23.7	53.4	3.8	-39.5	38.4	37.8	31.1
14	7.2	-2.4	19.6	1.0	56.7	14.5	-38.9	25.6	41.1	40.6
15	14.6	-59.6	9.0	1.3	44.1	30.5	-32.5	10.7	27.3	30.5
16	16.2	-36.0	11.2	41.7	27.3	21.8	-11.0	21.6	33.4	29.4
17	25.0	-20.6	7.0	2.8	30.0	4.7	-9.3	20.2	34.7	29.7
18	7.6	10.2	1.5	1.0	51.4	17.6	-2.9	16.0	42.5	29.2

Appendix Table 22 Daily lymphocyte counts expressed as the percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Days after inoculation	Sheep No. 135	137	154	981	993	64	91	92	152	153
1	2.1	0.1	4.0	2.0	0.3	2.0	1.6	2.8	5.4	4.6
2	-16.3	-23.5	-0.2	3.8	-0.2	-4.5	-17.0	-3.5	24.3	-30.0
3	-28.5	-66.8	-18.6	8.2	-11.6	-31.5	-37.0	-4.2	-3.2	-39.5
4	-42.8	-76.2	-56.7	6.3	-11.3	-48.7	-35.6	-20.7	-58.6	-70.7
5	-69.6	-55.0	-51.8	0.8	-23.6	-48.4	-59.0	-31.0	-67.2	-75.7
6	-68.6	-50.2	-62.7	-19.0	-19.7	-46.0	-63.0	-39.0	-57.7	-68.5
7	-66.0	-51.3	-56.4	-43.0	-20.2	-44.4	-75.0	-54.0	-60.0	-66.7
8	-30.0	-69.2	-65.4	-45.0	-35.0	-63.8	-52.0	-40.0	-50.0	-70.2
9	-45.0	-64.0	-53.5	-68.0	-22.2	-49.0	-44.4	-37.0	-44.0	-61.5
10	-62.4	-67.8	-60.0	-49.6	-44.4	-39.4	-47.0	-36.8	-33.0	-49.0
11	-67.3	-59.5	-40.6	-46.0	-39.0	-37.4	-33.7	-36.5	-14.0	-58.7
12	-51.7	-62.6	-17.3	-57.3	-37.5	-60.7	-27.7	-35.0	-17.0	-63.8
13	-34.0	-60.0	-15.0	-32.5	-47.3	-55.0	-37.0	-34.0	-13.7	-57.8
14	-50.4	-44.0	-28.5	-20.4	-43.3	-49.0	-20.0	-33.8	-35.7	-50.2
15	-5.5	-52.5	-36.8	-32.0	-24.2	-45.0	-9.2	-33.6	-25.6	-43.0
16	-12.0	-46.9	-30.2	-12.3	-16.7	-34.0	-15.7	-19.5	-16.0	-41.6
17	-16.0	-40.4	-32.4	-8.8	-22.0	-24.4	-4.2	-12.0	-16.7	-45.0
18	-27.5	-29.2	10.0	5.0	-21.0	-13.2	-8.7	-6.8	-15.7	-20.0

Appendix Table 23 Daily neutrophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Days after inoculation	Sheep No. 135	137	154	981	993	64	91	92	152	153
1	16.0	16.7	5.3	0.7	4.2	2.0	3.7	7.2	21.3	11.0
2	33.4	5.2	3.7	11.7	21.0	2.0	49.5	25.3	13.7	10.0
3	44.7	-30.0	26.6	13.7	17.0	8.0	60.0	19.6	17.7	10.2
4	-32.5	-19.3	17.8	5.6	-5.0	12.7	65.0	11.0	46.5	17.5
5	-16.0	-15.4	7.0	1.8	-30.2	14.8	40.5	11.8	7.6	4.0
6	-39.3	-10.3	31.5	19.0	-33.6	19.4	40.5	-21.5	7.0	-7.6
7	-1.2	-14.0	17.6	14.4	-37.6	30.2	13.5	-49.7	-9.0	-19.0
8	-2.0	-0.4	2.8	18.4	-34.6	-32.7	32.7	-40.8	-20.0	-12.0
9	-0.04	-1.6	-37.0	20.3	-37.3	-69.5	-4.0	-43.1	-27.0	-16.8
10	-5.0	-10.0	-1.5	-26.4	-63.2	-56.0	-8.3	-63.3	-29.8	-52.2
11	-35.8	-40.8	-1.8	-6.3	-65.2	-50.4	-19.7	-67.0	-74.4	-24.6
12	-59.6	-41.7	-27.0	-7.3	-39.3	-40.0	-9.7	-69.3	-69.2	-0.01
13	-71.2	-57.5	-20.4	-24.0	-17.0	-41.7	-13.0	-49.6	-63.0	-8.3
14	-61.0	-49.7	-64.6	-37.0	-54.0	-46.0	-20.4	-45.0	-72.0	-47.7
15	-39.0	-47.0	-70.6	-45.5	-41.7	-40.9	-19.2	-49.0	-78.7	-57.8
16	-35.4	-53.0	-35.0	-35.0	-40.6	-18.6	-19.4	-48.8	-75.8	-72.0
17	-36.8	-57.0	12.7	1.0	-50.3	9.4	-1.5	-48.6	-81.0	-53.6
18	-12.8	-18.6	12.3	3.0	-34.0	11.6	37.5	-37.8	-20.7	-59.3

Appendix Table 24 Daily eosinophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Days after inoculation	Sheep No. 135	137	152	153	154	981	993	64	91	92
1	16.0	-25.0	9.4	33.4	-17.8	-3.4	0.5	-48.0	0.2	4.4
2	-88.0	-28.8	35.4	38.2	-34.3	-48.7	60.0	-34.7	0.2	48.0
3	-90.0	-71.8	35.0	22.4	-26.6	-47.0	-52.0	-52.6	41.5	42.3
4	-100	-100	-45.0	-84.0	-80.7	-100	-100	-100	50.5	-19.5
5	-100	-100	-42.0	-55.7	-81.4	-100	-100	-100	4.5	-58.5
6	-71.2	-100	-100	-71.7	-72.0	-77.0	-100	-69.0	4.5	-30.4
7	-100	-81.3	-100	-85.0	-100	-68.5	-100	-68.6	-54.4	-0.2
8	-100	-82.3	-100	-100	-100	-100	-12.5	-100	-100	-100
9	-100	-82.5	-79.5	-100	-100	-100	-22.8	-100	-100	-100
10	-87.8	-100	-79.0	-100	-69.0	-100	-9.3	-100	-100	-21.0
11	-71.3	-90.0	-77.0	-46.0	-85.0	-66.8	-10.0	-100	-100	-100
12	-69.8	-90.0	-77.0	-100	-82.8	-73.7	-24.5	-77.8	22.3	-100
13	-54.0	-73.0	-76.0	-8.3	22.4	27.0	-19.7	-76.0	31.0	30.5
14	29.3	26.8	-64.5	-100	26.0	17.0	-8.8	-74.3	22.7	22.8
15	20.6	21.3	16.6	-64.4	24.0	16.3	-32.4	32.4	21.5	23.8
16	25.6	15.4	12.6	24.0	12.8	14.0	-27.0	4.1	15.5	24.2
17	23.0	18.0	15.0	10.3	10.7	8.6	4.8	16.6	19.0	24.7
18	22.0	13.0	14.0	1.7	14.4	8.0	9.5	17.2	8.0	15.0

Appendix Table 25 Daily monocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated simultaneously with TBF-infected blood 10^{-3} and PI-3 virus

Days after inoculation	135	137	152	153	154	981	993	64	91	92
1	-0.5	-50.0	-17.1	-24.7	-7.0	-51.7	-53.1	-65.5	-22.6	-41.2
2	34.9	-39.0	-39.6	-52.2	7.8	-48.7	-13.7	-34.7	-10.9	-25.4
3	42.4	-69.0	-53.7	-35.5	27.5	-46.9	-12.0	-36.8	12.6	-8.7
4	40.5	-23.0	-17.5	-19.1	37.1	2.5	-42.3	-14.2	19.6	-9.6
5	30.3	-23.8	31.4	-21.6	35.1	4.9	-14.2	0.8	19.8	-34.2
6	22.8	-6.8	34.5	-29.2	43.1	1.2	-7.4	5.7	22.1	-35.5
7	21.1	-2.7	36.2	0.7	55.4	26.5	-13.7	24.6	25.9	-20.6
8	39.4	-14.4	33.4	4.3	38.0	23.1	42.8	24.1	11.5	-19.7
9	22.8	0.7	6.8	42.3	49.8	30.9	43.1	8.2	18.9	30.0
10	13.8	1.0	11.3	7.4	41.6	43.9	3.8	14.1	41.5	26.4
11	27.3	1.2	27.6	11.9	39.5	41.2	11.1	17.6	38.6	35.7
12	51.8	3.9	27.7	27.5	40.0	40.6	5.4	1.4	41.4	34.8
13	37.1	14.5	6.4	7.4	42.6	2.5	1.6	8.4	40.6	47.3
14	38.8	15.9	0.9	12.2	26.6	25.1	0.5	3.3	17.0	14.9
15	29.0	17.0	3.3	4.9	24.4	41.1	9.7	1.4	2.3	20.2
16	33.3	0.5	11.1	16.3	11.8	48.7	2.7	21.5	22.1	15.2
17	48.9	1.2	3.0	4.3	36.8	42.0	5.4	17.8	22.6	15.5
18	47.7	12.4	23.8	17.2	38.7	44.7	15.8	15.4	3.0	13.9

Appendix Table 26 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Days after inoculation	Sheep No. 139	140	163	164	95	96	109	113	115	116
1	0.7	1.0	0.7	0.3	14.8	13.3	5.4	10.8	10.0	1.6
2	-27.5	3.0	-7.8	15.7	6.8	-20.5	8.5	-3.4	-10.0	-9.0
3	-43.8	6.2	-16.0	-56.0	-11.0	-23.5	-30.6	-16.0	-39.5	-25.5
4	-60.2	-47.0	-53.5	-42.2	-23.4	-50.7	-42.4	-25.0	-61.0	-38.8
5	-68.6	-68.6	-60.5	-23.0	-20.5	-58.4	-37.0	-57.0	-67.5	-49.5
6	-66.0	-61.0	-64.8	-33.8	-51.0	-62.9	-44.0	-49.0	-62.6	-53.0
7	-73.0	-62.3	-55.6	22.4	-41.5	-63.2	-49.0	-48.6	-64.3	-63.3
8	-77.2	-68.8	-57.4	-37.4	-43.2	-57.0	-46.4	-55.4	-67.8	-53.0
9	-77.0	-67.5	-44.8	-27.2	-54.0	-54.3	-39.5	-56.2	-68.5	-58.5
10	-64.0	-59.0	-37.3	-30.6	-47.8	-42.4	-55.3	-33.0	-64.6	-25.5
11	-70.7	-57.6	-32.3	-29.6	-47.0	-47.5	-50.4	-12.0	-59.6	-14.4
12	-68.7	-43.3	-33.8	-27.3	-36.0	-	-26.0	-3.0	-59.8	-0.5
13	-66.6	-50.3	-31.2	-25.8	-36.0	-	-27.7	2.0	-57.5	-0.5
14	-59.0	-58.8	-36.2	-13.0	-32.9	-	-24.8	5.0	-61.0	-15.0
15	-41.4	-49.0	-31.0	9.0	-26.2	-	-7.3	11.0	-51.4	-3.0
16	-42.3	-40.0	-25.8	6.1	3.0	-	12.3	15.2	-22.4	-3.2
17	-36.0	-31.4	-17.3	8.8	5.0	-	11.5	20.3	-32.0	-5.0
18	6.7	-30.0	-18.6	12.0	10.0	-	14.0	11.4	-23.3	-3.4

Appendix Table 27 Daily neutrophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} and PI-3 virus at the onset of the TBF parasitaemia

Days after inoculation	Sheep No. 139	140	163	164	95	96	109	113	115	116
1	4.0	1.0	19.8	5.2	7.0	19.2	4.4	3.3	14.2	9.4
2	-14.0	18.8	22.0	2.6	4.3	5.5	11.2	25.3	13.2	5.3
3	-20.3	12.7	27.0	32.0	9.3	9.1	34.8	39.3	6.5	23.0
4	-43.6	58.6	33.4	8.8	10.5	12.7	34.7	45.0	12.6	8.7
5	-66.3	4.7	26.4	18.5	12.0	8.7	24.0	35.3	-0.4	1.6
6	-47.8	26.0	35.2	36.0	11.7	0.2	19.3	31.0	-5.8	-2.5
7	-37.0	-31.4	23.0	34.3	-4.3	-2.3	23.8	38.4	-0.5	-4.0
8	-48.8	-29.2	4.0	29.0	-32.3	-25.5	0.4	37.7	-25.0	-3.8
9	-53.0	-32.7	-41.2	-53.0	-27.3	-34.7	1.0	3.6	-24.8	-9.0
10	-72.3	-12.8	-53.0	-57.3	-64.7	-61.0	-10.2	-31.3	-66.4	-43.7
11	-47.2	-21.0	-55.4	-55.8	-74.0	-65.0	-35.5	-10.6	-61.0	-30.7
12	-27.6	-45.4	-62.3	-38.0	-76.6	-	-41.2	-30.5	-61.0	-38.0
13	-76.0	-6.8	-58.0	-31.5	-73.0	-	-48.2	-41.8	-65.7	-38.0
14	-67.0	-7.0	-54.6	-17.2	-63.6	-	-23.0	-17.8	-64.7	-30.4
15	-74.2	-73.3	-24.4	-35.5	-58.4	-	-31.2	-8.0	-58.3	-40.2
16	-56.0	-59.0	-47.0	-2.0	-8.8	-	-7.3	-19.0	-7.6	-22.7
17	-57.0	-59.6	-36.5	-3.8	0.4	-	0.5	-1.5	-22.3	-20.0
18	-33.8	-21.5	-21.0	4.7	1.0	-	1.0	-18.6	-10.0	-20.7

Appendix Table 28 Daily eosinophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Days after inoculation	Sheep No. 139	140	163	164	95	96	109	113	115	116
1	19.2	1.1	-49.0	-33.3	61.0	55.5	50.0	-40.0	-10.0	41.6
2	59.7	35.6	-56.0	-61.4	-36.0	-11.0	74.7	-56.2	-19.3	69
3	24.2	9.1	-52.8	-100	-36.0	-55.7	37.5	-69.0	-65.0	28.7
4	-6.3	-100.0	-61.6	-67.4	-37.0	-100.0	-100.0	-15.6	-72.0	-100
5	-32.0	-100.0	-100.0	-100.0	-37.2	-100.0	-100.0	-0.2	-74.5	-100
6	-32.0	-100.0	-100.0	-100.0	-0.2	-100.0	-100.0	-51.0	-71.4	-100
7	-100.0	-100.0	-100.0	-100.0	-100.0	-68.4	-17.4	-100	-71.5	-0.2
8	-100.0	-79.0	-100.0	-62.5	-100.0	-68.8	-64.0	-15.7	-100	-17
9	-50.2	-36.3	-55.0	-69.2	-100.0	-69.0	-62.6	-100	-100	-57
10	-46.0	-47.6	-67.7	28.0	-66.8	-69.7	-70.8	-100	-100	-57
11	-47.5	8.0	-66.8	17.2	-100	38.7	17.0	-100	-72.7	-22.6
12	-55.3	17.6	1.8	18.6	-31.3	59.6	53.8	12.3	-33.4	52
13	-50.4	1.7	27.4	18.6	-5.2	-	67.4	4.6	-40	31
14	-37.3	6.6	41.3	24.0	28.6	-	75.5	10.2	-38	70
15	-43.6	18.0	7.3	1.5	7.8	-	28.2	25.4	-62	79
16	16.9	20.7	18.0	10.2	4.4	-	9.6	5.1	-52	66
17	22.3	0.5	18.0	10.2	45.0	-	7.6	12.8	-53	46
18	5.5	15.0	19.8	9.8	11.0	-	2.0	1.1	-58	48

Appendix Table 29 Daily monocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} and with PI-3 virus at the onset of the TBF parasitaemia

Days after inoculation	Sheep No. 139	140	163	164	95	96	109	113	115	116
1	-46.6	-24.3	-49.0	-33.6	-19.8	-44.3	-43.3	-13.6	-44.6	-14.2
2	-31.5	-24.0	-56.0	56.9	19.2	45.4	-17.2	-18.0	32.6	-54.6
3	-16.5	-19.8	-4.2	39.0	24.5	43.8	-2.9	-10.3	52.7	-10.0
4	-46.6	-38.2	-67.7	52.2	50.6	44.6	-4.1	-34.9	55.4	-9.2
5	3.2	-26.4	26.4	61.4	60.7	49.1	-21.8	-32.7	35.7	-16.8
6	31.6	-18.3	21.0	41.2	67.4	50.6	-19.9	-14.7	50.8	-5.0
7	44.4	-10.9	34.9	65.0	71.0	67.6	-17.0	-23.7	42.3	59.7
8	35.0	3.4	29.8	64.3	67.7	63.9	-9.2	12.4	60.2	54.0
9	39.0	3.7	41.6	57.6	71.0	66.8	10.2	27.3	56.2	60.9
10	15.5	6.1	28.4	45.7	75.8	48.6	10.4	48.5	44.0	69.8
11	1.9	1.1	46.6	60.4	61.2	55.8	23.1	52.3	51.2	40.7
12	34.3	5.3	50.0	63.3	53.4	68.7	4.8	50.5	30.0	40.7
13	40.9	4.2	51.5	57.1	68.0	-	8.2	42.8	54.0	41.0
14	40.1	5.8	40.0	49.4	74.2	-	29.5	25.6	51.5	57.6
15	46.0	6.1	44.2	60.9	65.5	-	27.1	15.2	65.8	47.5
16	31.7	1.4	42.4	63.8	72.4	-	21.4	7.5	57.1	64.5
17	34.1	9.8	34.1	64.2	73.5	-	11.8	7.1	64.8	48.2
18	46.4	14.2	36.4	40.1	72.4	-	18.9	7.5	57.8	59.3

Appendix Table 30 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Days after inoculation	Sheep No. 68	984	990	78	79	992	797	62	101	103
1	7.8	10.6	6.6	10.5	14.7	3.8	4.0	8.7	15.0	16.0
2	13.3	2.6	10.2	-14.0	19.8	6.8	4.0	-50.0	-6.7	-24.0
3	-48.8	6.0	6.0	-1.8	12.0	23.0	15.8	-26.0	-23.0	-42.2
4	-54.2	9.4	24.0	-49.0	-43.2	-22.4	-8.6	-38.0	-71.0	-56.2
5	-62.6	-12.4	-0.1	-45.4	-59.2	-5.8	-36.6	-34.8	-53.2	-65.2
6	-48.7	-2.3	-0.2	-29.0	-65.4	-4.0	-57.7	-74.0	-56.3	-70.7
7	-66.0	-31.7	-5.0	-31.0	-54.2	-20.6	-61.8	-73.0	-59.0	-76.7
8	-44.0	-46.2	-6.1	-14.5	-53.2	-0.3	-50.4	-44.5	-54.8	-59.3
9	-42.3	-65.7	-45.4	-41.8	-58.0	-24.3	-45.3	-70.6	-48.0	-55.6
10	-16.7	-52.0	-36.6	-9.0	-33.3	-32.5	-0.07	-66.3	-35.2	-51.2
11	-28.0	-8.2	-12.7	-18.0	-6.2	-26.4	-28.0	-78.3	-43.0	-30.0
12	-15.0	-23.0	16.0	-8.0	-13.5	-38.5	-14.6	-77.0	-13.0	-50.0
13	-1.0	-10.0	16.6	-21.8	-34.5	-48.0	-24.7	-75.0	-13.0	-18.0
14	-11.0	-19.4	9.0	-16.3	6.8	-29.8	-21.0	-53.2	-10.2	-20.2
15	-18.0	-30.0	11.8	-36.3	8.0	39.7	-27.8	-58.7	-4.0	8.0
16	-12.8	-18.2	0.6	-16.3	11.0	35.0	-24.7	-43.5	-4.2	5.4
17	-10.2	-29.7	27.2	22.5	16.5	43.8	-33.6	-51.0	-4.4	3.3
18	-1.2	-27.0	4.4	24.6	18.2	40.5	-15.0	-69.5	12.0	14.5

Appendix Table 31 Daily neutrophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Days after inoculation	Sheep No. 68	984	990	78	79	992	797	62	101	103
1	35.5	17.0	9.0	9.3	9.0	47.6	17.6	4.0	1.4	3.5
2	33.3	43.8	21.5	9.4	-47.2	26.6	8.0	-62.0	4.3	10.0
3	28.8	70.1	21.2	22.5	-47.2	79.0	30.4	-80.0	-0.7	16.6
4	1.0	70.0	36.2	29.0	-60.0	48.0	46.3	-70.0	-57.0	-45.0
5	-22.6	69.0	29.6	18.4	-29.0	63.7	27.2	-83.5	-52.2	-12.0
6	-56.0	72.6	20.0	28.0	-56.0	54.4	10.3	-70.0	-33.5	-4.3
7	-45.0	-61.8	17.5	-42.5	-60.0	-47.8	11.3	-87.0	-30.6	-37.0
8	-47.4	-53.8	3.0	-25.8	-65.4	-10.0	-26.5	-71.0	-28.7	-42.7
9	-66.6	-1.7	-9.0	-32.2	-67.2	-9.0	-60.6	-80.0	-42.5	-59.0
10	-77.3	-41.0	-63.0	-61.2	-89.0	-19.2	-66.7	-85.0	-53.2	-57.2
11	-85.0	-49.5	-62.7	-90.5	-96.3	-31.8	-90.2	-78.0	-51.5	-52.3
12	-68.0	-25.0	-1.0	-80.6	-94.5	-51.8	-75.0	-80.0	-65.5	-68.5
13	-78.6	-29.2	-25.7	-87.0	-89.0	-37.8	-50.0	-75.0	-65.2	-68.5
14	-66.0	-42.0	-20.0	-93.0	-49.0	30.2	-79.0	-89.0	-0.01	-50.0
15	-64.0	-54.7	-36.3	-51.6	-60.0	26.6	-47.0	-79.0	-20.4	-23.0
16	-60.0	-41.4	-18.0	-32.2	-58.0	31.2	-38.2	-84.0	-13.0	-10.0
17	-57.7	30.7	-10.0	-38.7	-58.0	38.8	-14.7	-83.0	-18.6	20.5
18	-62.2	30.7	61.4	-48.3	-60.0	62.0	-11.7	-54.0	-16.6	26.5

Appendix Table 32 Daily eosinophil counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Days after inoculation	Sheep No. 68	984	990	78	79	992	797	62	101	103
1	1.5	3.0	25.3	22.7	65.3	43.5	23.2	1.0	62.3	22.8
2	48.6	64.6	-34.0	48.5	54.0	-65.0	26.0	54.4	-54.2	66.0
3	-60.1	73.7	-41.0	-52.4	-50.0	-27.0	22.2	50.2	-57.8	-63.0
4	-100	84.0	-100	-3.0	-75.0	-65.4	-100	33.6	-82.0	-75.0
5	-100	75.7	-100	-3.0	-100	-41.5	-100	0.5	-100	-100
6	-100	45.6	-100	-22.3	-100	-100	-100	37.2	-100	-100
7	-37.5	22.2	-100	-100	-100	-100	-100	-54.7	-100	-100
8	-47.3	-50.7	-1.0	-100	-84.2	-100	-41.2	-50.0	-100	-19.8
9	-43.6	-100	-1.0	-100	-75.0	-69.5	-47.5	-100	-15.7	-19.8
10	-70.3	-100	-77.2	-100	-83.0	-54.3	-81.8	-100	-13.5	-71.0
11	-74.4	-100	-70.4	-100	-78.7	-60.2	-41.2	-100	-39.2	-66.0
12	-66.1	-40.4	-52.2	-61.6	-78.2	-57.8	33.0	-100	-72.0	-75.8
13	-64.0	-42.0	-44.3	-75.2	-83.5	-45.6	28.5	-100	-23.5	-64.5
14	32.0	-50.7	36.6	-51.4	-69.0	-54.3	20.5	-7.5	48.9	-60.0
15	50.0	-4.7	6.3	-47.5	-40.0	4.0	27.7	3.8	65.7	40.2
16	4.6	10.0	12.0	-4.8	-75.0	12.7	45.0	0.5	30.6	55.5
17	8.0	10.0	26.6	-4.0	-70.0	14.5	46.0	17.0	33.3	56.0
18	12.0	19.2	24.0	6.0	-50.0	12.0	47.0	19.4	34.8	26.7

Appendix Table 33 Daily monocyte counts expressed as percentages of the pre-inoculation value in ten lambs inoculated with TBF-infected blood 10^{-1} alone

Days after inoculation	Sheep No. 68	984	990	78	79	992	797	62	101	103
1	-10.0	8.8	-13.4	-21.2	-12.2	-11.4	-18.5	-5.0	-9.5	-18.4
2	-11.6	1.1	24.7	-12.5	75.9	-17.3	-13.8	-5.0	-9.0	70.4
3	-12.0	26.0	75.8	-18.7	30.0	-3.5	-17.8	-7.5	40.5	31.2
4	-20.0	60.4	87.9	-15.0	76.6	-5.7	-24.2	-7.5	28.9	32.5
5	-15.7	14.7	75.0	-6.2	68.1	-12.2	-29.8	-20.0	61.9	62.5
6	-24.2	61.7	86.9	-15.0	65.0	62.0	-7.8	-20.6	60.4	75.4
7	-26.0	34.3	73.8	-1.3	68.8	21.3	-2.4	-26.8	63.3	65.9
8	-28.4	55.8	88.7	-12.0	68.4	60.6	-7.7	-15.0	46.4	40.7
9	-18.0	22.2	69.7	-21.1	82.5	60.1	-16.0	-18.2	13.2	66.0
10	-12.0	60.6	45.5	-32.2	82.5	2.5	-19.7	-15.0	7.4	65.9
11	-0.4	43.1	51.6	-36.6	80.0	44.1	-3.1	-15.0	23.6	63.8
12	-8.7	49.6	73.8	-36.6	80.0	6.5	-1.9	-12.5	21.6	40.7
13	-13.3	-25.1	75.7	-27.7	72.0	65.0	-0.7	-15.0	34.6	60.3
14	-11.5	-28.8	81.0	-27.5	80.0	2.5	-2.9	1.2	48.9	79.5
15	-10.4	-2.2	68.5	-15.0	76.6	35.9	-1.1	20.0	22.7	77.6
16	-10.0	-8.8	60.8	-18.6	77.2	57.7	8.8	2.4	47.7	64.3
17	1.6	24.0	47.5	17.3	78.4	54.0	0.1	0.7	4.9	3.4
18	1.3	4.3	47.3	17.5	73.4	25.9	0.3	11.2	13.9	16.0

Appendix Table 34 Means and standard deviations of the febrile reactions (°C) in eight TBF-infected lambs and the eight non-infected siblings

Days after inoculation	TBF-infected lambs		Non-infected lambs	
	Mean	Standard deviation	Mean	Standard deviation
0	39.7	0.4	39.8	0.4
1	39.7	0.4	39.8	0.3
2	39.7	0.5	39.9	0.2
3	41.0	0.5	40.0	0.1
4	41.1	0.5	39.9	0.3
5	41.3	0.5	39.9	0.3
6	40.9	0.4	39.9	0.3
7	40.7	0.4	39.8	0.3
8	40.5	0.4	39.8	0.3
9	40.4	0.6	39.9	0.3
10	40.4	0.8	39.9	0.2
11	40.1	0.4	39.8	0.3
12	39.7	0.3	39.8	0.2
13	39.8	0.4	39.9	0.2
14	39.7	0.4	39.8	0.3

Appendix Table 35 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in eight TBF-infected lambs

Days after inoculation	Lamb No. 60	66	8	147	82	103	152	143
1	-35.0	3.5	-0.3	0.7	-20.6	2.6	4.6	3.1
2	-52.9	2.3	-28.7	3.3	-34.5	3.7	9.0	0.3
3	-46.3	0.6	-45.6	-26.7	-60.8	-45.9	-19.4	16.7
4	-52.6	10.3	-50.9	-30.8	-80.7	-79.9	-22.0	13.0
5	-64.7	-33.0	-50.0	-42.3	-77.9	-76.6	-25.7	11.5
6	-43.9	-32.8	-37.8	-13.8	-75.4	-73.0	-68.9	-27.0
7	-46.9	-33.6	-33.5	-5.4	-79.4	-74.6	-72.0	-25.8
8	-31.8	-20.0	-26.0	18.3	-70.3	-55.4	-56.0	-15.9
9	-40.6	-19.8	-16.5	38.8	-63.6	-34.4	-36.6	-18.8
10	-26.0	- 3.2	- 2.4	36.4	-63.1	-46.7	-39.7	-17.0
11	-34.7	-16.2	-18.9	27.7	-64.3	-51.9	-65.3	-16.2
12	-26.0	- 1.5	- 2.9	41.6	-71.7	-56.2	-63.4	-18.0
13	8.0	7.8	- 7.4	35.6	-70.8	-59.2	-68.0	37.2
14	5.3	30.6	-22.0	18.8	-68.0	-62.8	-65.7	30.4

Appendix Table 36 Daily neutrophil counts expressed as percentages of the pre-inoculation value in eight TBF-infected lambs

Days after inoculation	Lamb No. 60	66	8	147	82	103	152	143
1	41.0	20.4	6.0	2.4	- 7.3	- 7.5	5.9	5.3
2	39.8	26.0	5.9	28.6	-37.0	-15.0	7.6	2.7
3	9.5	1.9	2.7	62.2	-33.2	- 3.9	0.6	20.3
4	14.0	13.6	20.0	59.7	-40.0	-16.7	-41.4	32.4
5	29.4	49.1	21.9	61.3	-26.9	-15.5	-41.6	50.2
6	48.0	42.5	30.0	40.9	-18.3	-10.6	-44.2	48.3
7	48.9	33.3	21.2	29.0	- 6.4	- 4.4	-22.7	63.6
8	-64.2	-49.2	- 8.7	45.0	-49.8	-37.5	-44.8	32.7
9	-43.9	-55.0	-41.2	- 1.6	-85.0	-78.9	-77.4	-51.2
10	-51.4	-57.4	-67.2	-65.5	-84.9	-85.3	-71.7	-53.2
11	-61.3	-67.3	-52.4	-76.9	-87.9	-91.0	-87.9	-58.4
12	-60.2	-61.8	-44.5	-50.4	-65.5	-83.7	-68.3	- 2.7
13	-52.7	-66.3	-66.7	-60.0	-62.4	-76.2	-66.0	7.2
14	-16.8	-26.5	-65.4	-40.2	-75.0	-70.5	-65.7	9.7

Appendix Table 37 Daily eosinophil counts expressed as percentages of the pre-inoculation value in eight TBF-infected lambs

Days after inoculation	Lamb No. 60	66	8	147	82	103	152	143
1	42.6	8.2	1.7	50.8	17.4	66.6	1.6	-50.4
2	7.8	26.7	83.0	66.7	23.6	0.5	37.7	-75.6
3	-38.4	53.6	-100.0	-27.0	30.0	26.9	-79.4	-77.2
4	-62.0	58.0	- 33.6	-29.8	-40.4	-100.0	-100.0	-100.0
5	-100.0	-100.0	-100.0	-26.0	-100.0	- 19.6	-100.0	-100.0
6	- 79.3	-100.0	- 48.5	-35.0	-100.0	-100.0	-100.0	-100.0
7	-100.0	- 7.0	- 10.9	-77.7	-100.0	-100.0	-100.0	- 5.7
8	- 23.2	- 85.0	- 8.1	-100.0	- 24.0	- 4.8	- 84.2	-100.0
9	- 36.0	- 25.8	- 78.6	-100.0	- 32.0	- 43.0	- 82.0	-100.0
10	- 51.3	-100.0	- 80.8	-100.0	-100.0	-100.0	-100.0	-100.0
11	- 16.0	- 13.8	- 59.7	- 56.8	- 69.6	- 60.2	-100.0	-100.0
12	- 23.3	- 68.4	-100.0	-100.0	- 69.0	-100.0	-100.0	-100.0
13	-100.0	-100.0	- 72.5	78.5	- 69.0	- 67.7	-100.0	-100.0
14	- 34.4	- 53.9	-100.0	5.0	- 6.7	- 27.8	- 77.2	12.3

Appendix Table 38 Daily monocyte counts expressed as percentages of the pre-inoculation value in eight TBF-infected lambs

Days after inoculation	Lamb No. 60	66	8	147	82	103	152	143
1	42.6	54.3	0.8	-33.9	-17.4	-50.0	- 1.9	0.0
2	40.7	59.0	20.7	37.6	-34.8	-49.8	-52.0	49.5
3	2.6	53.0	62.9	81.0	- 4.5	-31.8	-38.2	64.0
4	32.6	16.5	39.4	84.7	-70.2	-25.0	-58.7	76.8
5	40.0	84.6	68.0	80.3	30.0	-17.0	-68.0	48.2
6	59.5	91.8	89.7	86.5	70.0	-56.0	-63.9	90.6
7	66.6	84.5	84.0	80.5	70.0	-51.0	0.8	86.8
8	69.3	81.3	87.0	90.5	67.0	68.4	15.4	86.9
9	59.9	73.0	83.5	74.6	67.4	74.8	38.5	85.5
10	53.7	69.8	83.9	82.2	- 7.0	54.5	23.3	78.7
11	49.8	69.0	73.0	74.0	- 8.9	64.0	- 7.0	86.9
12	18.4	63.9	58.3	86.5	- 8.7	8.8	- 8.0	67.5
13	-73.9	37.3	72.3	71.8	- 9.0	- 4.7	- 4.5	78.0
14	-41.6	13.6	65.8	43.3	- 7.6	- 7.8	- 8.7	83.6

Appendix Table 39 Daily lymphocyte counts expressed as percentages of the pre-inoculation value in the eight non-infected lambs

Days after inoculation	Lamb No. 59	67	7	148	81	102	153	142
1	-24.0	-19.3	0.4	3.6	-11.5	9.5	- 5.6	11.6
2	-24.6	-15.5	5.8	0.8	-14.3	10.0	- 1.9	19.8
3	- 4.5	- 2.3	5.4	-10.7	-24.8	0.2	- 6.8	14.3
4	- 1.7	-36.7	3.4	-12.0	-25.4	2.4	-10.9	14.7
5	0.4	- 5.0	12.5	- 9.8	-25.3	2.9	-12.2	14.5
6	9.8	3.9	2.8	-18.0	-26.0	- 2.0	-14.7	14.8
7	2.0	4.3	10.0	- 6.8	-30.5	- 4.0	-17.4	8.0
8	3.8	10.9	13.5	- 3.7	-33.0	-0.03	-20.3	10.8
9	8.2	34.3	11.5	-14.0	-38.3	- 4.6	-16.0	0.3
10	12.0	33.4	15.6	-12.9	-34.9	- 0.8	- 9.9	6.6
11	17.4	35.3	19.8	-11.9	-38.7	- 6.8	-15.6	1.5
12	14.0	33.7	29.0	- 8.3	-39.0	- 0.2	-17.0	3.4
13	14.6	37.9	21.7	-10.0	-12.0	- 5.3	-26.0	3.8
14	16.4	33.0	2.3	-10.5	-40.9	- 5.3	-28.4	3.3

Appendix Table 40 Daily neutrophil counts expressed as percentages of the pre-inoculation value in the eight non-infected lambs

Days after inoculation	Lamb No. 59	67	7	148	81	102	153	142
1	0.8	24.5	12.0	23.0	15.5	3.0	15.7	7.6
2	5.6	25.8	33.4	15.3	- 3.2	10.7	0.4	2.2
3	9.2	7.1	32.7	11.7	- 1.1	2.3	- 6.0	17.0
4	14.3	44.9	23.3	- 4.8	- 0.3	6.3	-11.4	7.2
5	13.0	35.4	32.0	- 0.9	-10.7	- 2.9	- 8.0	13.9
6	9.8	28.0	46.0	- 0.9	-14.6	- 2.5	- 2.2	7.2
7	3.5	25.0	48.6	-22.3	-19.8	- 4.9	-21.0	13.8
8	1.4	20.4	32.3	-12.7	-29.3	- 4.4	- 1.4	15.0
9	8.2	2.9	49.4	-28.3	-24.0	- 8.8	- 7.4	10.9
10	9.0	9.6	55.5	-24.2	-27.6	-14.7	-21.8	- 3.0
11	4.0	2.1	47.7	-26.5	-30.4	-0.08	-20.4	-0.03
12	5.3	6.0	70.2	20.5	-22.5	-10.8	- 9.9	- 1.4
13	5.6	18.2	48.9	3.8	-26.8	-16.2	- 7.2	- 4.4
14	12.4	29.5	45.7	21.0	-23.6	-14.0	-19.8	- 4.3

Appendix Table 41 Daily eosinophil counts expressed as percentages of the pre-inoculation value in the eight non-infected lambs

Days after inoculation	Lamb No. 59	67	7	148	81	102	153	142
1	50.4	32.4	5.9	-23.0	-28.4	6.8	-20.9	6.0
2	30.0	31.0	2.4	-23.3	-10.4	-35.0	-22.7	31.7
3	51.6	23.4	62.8	-27.0	- 2.9	-40.8	-27.6	12.5
4	2.9	25.1	66.0	28.8	-39.7	-42.4	-31.9	13.3
5	44.4	43.7	49.7	33.5	-42.3	-41.8	-64.9	13.0
6	32.0	51.8	48.7	14.0	-42.8	-22.8	-65.9	13.3
7	68.8	58.7	18.5	8.7	-46.3	-43.0	-36.8	28.6
8	52.7	34.3	65.9	9.3	-32.0	-42.7	-17.8	12.0
9	5.3	32.0	20.0	1.1	-34.5	-45.3	-33.9	4.8
10	34.0	42.8	26.9	8.9	-33.0	-44.7	-67.4	3.5
11	13.6	53.0	9.4	7.7	-68.9	-25.4	-52.0	0.2
12	11.5	43.0	13.7	2.7	-51.5	-63.0	-51.8	1.8
13	11.8	9.7	28.0	7.5	-34.6	- 9.5	-38.0	21.8
14	12.3	6.4	14.0	1.7	-36.0	-46.5	-42.8	20.8

Appendix Table 42 Daily monocyte counts expressed as percentages of the pre-inoculation value in the eight non-infected lambs

Days after inoculation	Lamb No. 59	67	7	148	81	102	153	142
1	-16.5	- 1.2	21.5	-48.7	4.5	-28.4	-25.7	6.0
2	-14.0	- 2.5	-49.0	-48.9	5.6	-27.9	-27.6	14.5
3	3.4	1.2	-71.2	-31.0	-17.6	- 1.2	-54.8	14.0
4	48.4	75.0	-72.3	-57.3	-19.4	-68.0	-78.7	42.0
5	38.3	72.0	-70.4	-55.7	-61.6	-67.7	-78.0	13.7
6	44.4	16.0	-71.0	-35.6	-23.6	- 3.5	-57.0	42.0
7	6.6	72.6	-69.3	-56.0	-28.4	-68.5	-60.5	10.9
8	5.7	79.5	- 5.9	-77.4	-32.0	-36.3	-79.5	12.0
9	52.8	63.3	-68.8	-59.6	-34.4	-39.3	-79.3	36.4
10	12.3	28.4	2.3	-17.9	-32.9	-38.5	-79.6	3.5
11	13.9	65.0	26.0	-17.0	-37.7	-37.8	-60.0	33.3
12	12.0	64.5	23.0	-18.0	-35.3	-38.3	-80.0	1.5
13	12.0	32.7	3.9	-18.8	- 2.0	-39.8	-42.0	2.0
14	12.7	77.5	13.0	-27.5	-26.0	-40.6	-64.0	34.0